BIBLIOGRAFI AFRICAN SWINE FEVER

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Bibliografi ini mencakup berbagai topik penting, antara lain:

- Struktur molekuler dan mekanisme interaksi protein ASFV, seperti pE301R, pA104R, dan p72.
- Pendekatan inovatif dalam pengembangan vaksin dan terapi, termasuk penggunaan teknologi CRISPR-Cas dan vaksin berbasis gen/protein.
- Analisis risiko dan epidemiologi, seperti siklus sylvatic ASFV di Kenya dan model penyebaran ASF pada populasi babi liar di Australia.
- Strategi diagnostik dan pengembangan alat deteksi cepat ASFV, seperti *immunochromatographic test strip* dan *digital PCR*.
- Upaya memahami mekanisme imunoevasi dan virulensi ASFV melalui penelitian protein MGF serta regulasi jalur interferon tipe I.

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Bogor, November 2024

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1. **Crystal structure of African swine fever virus pE301R reveals a ring-shaped trimeric DNA sliding clamp,**Jiqin Wu, Haixue Zheng, Peng Gong,Journal of Biological Chemistry,Volume 299, Issue 7,2023,104872,https://doi.org/10.1016/j.jbc.2023.104872.

Abstract:

African swine fever virus (ASFV) is an important animal pathogen that is causing a current African swine fever pandemic and affecting pork industry globally. ASFV encodes at least 150 proteins, and the functions of many of them remain to be clarified. The ASFV protein E301R (pE301R) was predicted to be a DNA sliding clamp protein homolog working as a DNA replication processivity factor. However, structural evidence was lacking to support the existence of a ring-shaped sliding clamp in large eukaryotic DNA viruses. Here, we have solved a high-resolution crystal structure of pE301R and identified a canonical ring-shaped clamp comprising a pE301R trimer. Interestingly, this complete-toroidal structure is different from those of the monomeric clamp protein homolog, herpes simplex virus UL42, and the C-shaped dimeric human cytomegalovirus UL44, but highly homologous to that of the eukaryotic clamp homolog proliferating cell nuclear antigen. Moreover, pE301R has a unique N-terminal extension that is important in maintaining the trimeric form of the protein in solution, while specific features in length and surface electrostatic potential of its interdomain connector implies specificity in interactions with binding partners such as the viral DNA polymerase. Thus, our data pave the way for further dissection of the processivity clamp protein structural and functional diversity and ASFV DNA replication mechanisms.

Keywords: African swine fever virus; E301R protein; DNA sliding clamp; DNA polymerase; clamp loader; crystal structure

2. **Recombinant porcine interferon cocktail delays the onset and lessens the severity of African swine fever,**Pengtao Jiao, Shuchao Wang, Wenhui Fan, He Zhang, Hongyan Yin, Yingli Shang, Hongfei Zhu, Wenjun Liu, Rongliang Hu, Lei Sun,Antiviral Research,Volume 215,2023,105644,https://doi.org/10.1016/j.antiviral.2023.105644.

Abstract:

African swine fever (ASF) is a highly contagious and deadly disease that affects domestic and wild pigs. No commercial vaccine or antiviral is currently available against ASF. The control of ASF primarily relies on implementing effective biosecurity measures during the breeding process. Here, we evaluated the preventive and therapeutic potential of the interferon (IFN) cocktail (a mixture of recombinant porcine IFN α and γ) on ASF. The IFN cocktail treatment delayed the onset of ASF symptoms and ASF virus (ASFV) replication for approximately one week. However,

IFN cocktail treatment could not prevent the death of the pigs. Further analysis showed that IFN cocktail treatment increased the expression of multiple IFN-stimulated genes (ISGs) in porcine peripheral blood mononuclear cells in vivo and in vitro. Additionally, IFN cocktail modulated the expression of pro- and anti-inflammatory cytokines and reduced tissue injury in the ASFV-infected pigs. Collectively, the results suggest that the IFN cocktail restricts the progression of acute ASF by inducing high levels of ISGs, contributing to the pre-establishment of antiviral status, and modulating the balance of pro- and anti-inflammatory mediators to lessen cytokine storm-mediated tissue damage.

Keywords: Recombinant porcine interferon; African swine fever virus; Interferon-stimulated gene; Inflammatory cytokine

3. **Epidemiology and ecology of the sylvatic cycle of African Swine Fever Virus in Kenya**,Vincent Obanda, Mercy Akinyi, Edward King'ori, Ruth Nyakundi, Griphin Ochola, Purity Oreng, Kevin Mugambi, Grace Mwihaki Waiguchu, Mary Chege, William Rosenbaum, Erik Bovinder Ylitalo, Anne Tuiskunen Bäck, Lisa Pettersson, Opanda Silvanos Mukunzi, Bernard Agwanda, Susanna Stenberg-Lewerin, Olivia Wesula Lwande,Virus Research,Volume 348,2024,199434,https://doi.org/10.1016/j.virusres.2024.199434.

Abstract:

African Swine Fever (ASF) is caused by a DNA virus (AFSV) maintained and transmitted by the Argasid ticks. The re-emergence of the disease in Africa coupled with its rapid spread globally is a threat to the pig industry, food security and livelihoods. The ecology and epidemiology of the ASFV sylvatic cycle, especially in the face of changing land use and land cover, further compounds the menace and impacts of this disease in Kenya. The study aimed to determine the occurrence and distribution of ASFV seroprevalence in warthog populations, the tick vectors and extent of tick infestation of warthog burrows, and the genotypes of ASFV in soft ticks in Kenya. Warthogs from different parts of Kenya were captured and venous blood was centrifuged to harvest sera. Warthog burrows were examined for their conditions and to extract ticks. Sera were analyzed for antibodies against ASFV using a commercial ELISA kit coated with p32 ASFV recombinant protein. Ticks were pooled, DNA extracted and the p72 gene of the ASFV was amplified by qPCR and conventional PCR. The overall seroprevalence of ASFV in warthogs was 87.5 %. A total of 228 warthog burrows were examined and 2154 argasid ticks were extracted from the burrows. Tick pools from Kigio Farm and Lewa Wildlife Conservancies were ASFV-positive by qPCR and conventional PCR. ASFV was further confirmed by the Twist Comprehensive Viral Research Panel (TCVRP), which also identified the argasid ticks as Ornithodoros porcinus. The ticks were infected

with virus genotype IX, and their occurrence overlaps with regions of previous ASF outbreaks in domestic pigs. Further, Viruses that could be tick endosymbionts/commensals or due to bloodmeal were detected in ticks by TCVRP; Porcine type-C oncovirus; Pandoravirus neocaledonia; Choristoneura fumiferana granulovirus; Enterobacteria phage p7; Leporid herpesvirus 4 isolate; 5; Human Lymphotropic virus; Human herpesvirus 5. In conclusion, our results suggest that infected Ornithodoros spp. seems to have a rich virome, which has not been explored but could be exploited to inform ASF control in Kenya. Further, the ecology of Ornithodoros spp. and burrow-use dynamics are complex and more studies are needed to understand these dynamics, specifically in the spread of ASFV at the interface of wild and domestic pigs. Further, our results provide evidence of genotype IX ASFV sylvatic cycle which through O. porcinus tick transmission has resulted in high exposure of adult common warthogs. Finally, the co-circulation of ASFV genotype IX in the same location with past ASF outbreaks in domestic pigs and presently in ticks brings to focus the role of the interface and ticks on virus transmission to pigs and warthogs.

Keywords: Tick-borne diseases; Microbial community; Food security; Soft ticks; Ornithodoros moubata; Argasid ticks

4. **African swine fever virus infection regulates pyroptosis by cleaving gasdermin A via active caspase-3 and caspase-4,**Shuai Li, Jie Song, Jia Liu, Shijun Zhou, Gaihong Zhao, Tingting Li, Li Huang, Jiangnan Li, Changjiang Weng,Journal of Biological Chemistry,Volume 300, Issue 6,2024,107307,https://doi.org/10.1016/j.jbc.2024.107307.

Abstract:

African swine fever, caused by the African swine fever virus (ASFV), is a viral hemorrhagic disease that affects domestic pigs and wild boars. ASFV infection causes extensive tissue damage, and the associated mechanism is poorly understood. Pyroptosis is characterized by the activation of inflammatory caspases and pore formation in the cellular plasma membrane, resulting in the release of inflammatory cytokines and cell damage. How ASFV infection regulates pyroptosis remains unclear. Here, using siRNA assay and overexpression methods, we report that ASFV infection regulated pyroptosis by cleaving the pyroptosis execution protein gasdermin A (GSDMA). ASFV infection activated caspase-3 and caspase-4, which specifically cleaved GSDMA at D75-P76 and D241-V242 to produce GSDMA into five fragments, including GSDMA-N1-75, GSDMA-N1-241, and GSDMA-N76-241 fragments at the N-terminal end of GSDMA. Only GSDMA-N1-241, which was produced in the late stage of ASFV infection, triggered pyroptosis and inhibited ASFV replication. The fragments, GSDMA-N1-75 and GSDMA-N76-241, lose the ability to induce pyroptosis. Overall ASFV infection differentially regulates pyroptosis by GSDMA in the

indicated phase, which may be conducive to its own replication. Our findings reveal a novel molecular mechanism for the regulation of pyroptosis.

Keywords: African swine fever; GADMA; caspase-3; caspase-4; pyroptosis

5. **The proteomic analysis uncovers the cellular responses to the African swine fever virus membrane proteins p54, p17, and pB117L,**Yuhong Chen, Jianqiang Ni, Chuanbin Wang, Xinyan Zhai, Tingrong Luo, Yi-Ping Li, Youchuan Wei, Yuliang Liu,Microbes and Infection,Volume 26, Issues 5–6,2024,105348,https://doi.org/10.1016/j.micinf.2024.105348.

Abstract:

African swine fever virus (ASFV) infection causes African swine fever (ASF), a highly contagious and fatal disease that poses severe threat to swine production. To gain insights into the host responses to ASFV, we generated recombinant adenovirus Ad5 expressing viral membrane proteins p54, p17, and pB117L individually and infected an alveolar cell line, 3D4/21, with these recombinant viruses. Then, the cell lysates were analyzed using label-free quantification proteomic analysis method. A total of 2158 differentially expressed proteins (DEPs) were identified, of which 817, 466, and 875 proteins were from Ad5-p54-, Ad5-p17-, Ad5-pB117L-infected 3D4/21 cells, respectively. Gene Ontology (GO) classification and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis revealed distinct yet interconnecting patterns of protein interaction networks. Specifically, the Ad5-p54 virus infection enriched the DEPs primarily involved in the metabolic pathways, endocytosis, adherens junction, and SNARE interactions in vesicular transport. The Ad5-p17 virus infection enriched the DEPs in endocytosis, ubiquitin-mediated proteolysis, N-Glycan biosynthesis, and apoptosis, while the Ad5-pB117L virus infection enriched the DEPs in metabolic pathways, endocytosis, oxidative phosphorylation, and focal adhesion. In summary, these results provide a comprehensive proteinomics analysis of the cellular responses to three ASFV membrane proteins, thus facilitating our understanding of ASFV pathogenesis.

Keywords: African swine fever virus; p54; p17; pB117L; Membrane protein; Proteomics

6. **Characterization of the monoclonal antibody and the immunodominant B-cell epitope of African swine fever virus pA104R by using mouse model,**Qichao Chen, Lixinjie Liu, Shibang Guo, Liang Li, Yifeng Yu, Zhankui Liu, Chen Tan, Huanchun Chen, Xiangru Wang,Microbiology Spectrum,Volume 12, Issue 3,2024, https://doi.org/10.1128/spectrum.01401-23.

Abstract:

The African swine fever virus (ASFV) structural protein pA104R is the only histone-like protein encoded by eukaryotic viruses. pA104R is an essential DNA-binding protein required for DNA replication and genome packaging of ASFV, which are vital for pathogen survival and proliferation. pA104R is an important target molecule for diagnosing, treating, and immune prevention of ASFV. This study characterized monoclonal antibodies (mAbs) against pA104R and found them to recognize natural pA104R in ASFV strains with different genotypes, showing high conservation. Confirmation analyses of pA104R epitopes using mAbs indicated the presence of immunodominant B-cell epitopes, and further characterization showed the high antigenic index and surface accessibility coefficients of the identified epitope. Furthermore, the pA104R protein functions through the polar interactions between the binding amino acid sites; however, these interactions may be blocked by the recognition of generated mAbs. Characterizing the immunodominant B-cell epitope of the ASFV critical proteins, such as pA104R, may contribute to developing sensitive diagnostic tools and vaccine candidate targets. IMPORTANCE African swine fever (ASF) is a highly pathogenic, lethal, and contagious viral disease affecting domestic pigs and wild boars. As no effective vaccine or other treatments have been developed, the control of African swine fever virus (ASFV) relies heavily on virus detection and diagnosis. A potential serological target is the structural protein pA104R. However, the molecular basis of pA104R antigenicity remains unclear, and a specific monoclonal antibody (mAb) against this protein is still unavailable. In this study, mAbs against pA104R were characterized and found to recognize natural pA104R in ASFV strains with different genotypes. In addition, confirmation analyses of pA104R epitopes using mAbs indicated the presence of immunodominant B-cell epitopes, and further characterization showed the high antigenic index and surface accessibility coefficients of the identified epitope. Characteristics of the immunodominant B-cell epitope of ASFV proteins, such as pA104R, may contribute to developing sensitive diagnostic tools and identifying vaccine candidate targets.

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Keywords: African swine fever virus; pA104R; monoclonal antibody; immunodominant B-cell epitope

7. **Testing multiplexed anti-ASFV CRISPR-Cas9 in reducing African swine fever virus,**Zezhong Zheng, Lei Xu, Yangbin Gao, Hongwei Dou, Yixuan Zhou, Xu Feng, Xiangjun He, Zhen Tian, Lingling Song, Guolong Mo, Jiapan Hu, Hongye Zhao, Hongjiang Wei, George M. Church, Luhan Yang, Clinton J. Jones,Microbiology Spectrum,Volume 12, Issue 7,2024,https://doi.org/10.1128/spectrum.02164-23.

Abstract:

African swine fever (ASF) is a highly fatal viral disease that poses a significant threat to domestic pigs and wild boars globally. In our study, we aimed to explore the potential of a multiplexed CRISPR-Cas system in suppressing ASFV replication and infection. By engineering CRISPR-Cas systems to target nine specific loci within the ASFV genome, we observed a substantial reduction in viral replication in vitro. This reduction was achieved through the concerted action of both Type II and Type III RNA polymerase-guided gRNA expression. To further evaluate its anti-viral function in vivo, we developed a pig strain expressing the multiplexable CRISPR-Cas-gRNA via germline genome editing. These transgenic pigs exhibited normal health with continuous expression of the CRISPR-Cas-gRNA system, and a subset displayed latent viral replication and delayed infection. However, the CRISPR-Cas9-engineered pigs did not exhibit a survival advantage upon exposure to ASFV. To our knowledge, this study represents the first instance of a living organism engineered via germline editing to assess resistance to ASFV infection using a CRISPR-Cas system. Our findings contribute valuable insights to guide the future design of enhanced viral immunity strategies.

IMPORTANCE

ASFV is currently a devastating disease with no effective vaccine or treatment available. Our study introduces a multiplexed CRISPR-Cas system targeting nine specific loci in the ASFV genome. This innovative approach successfully inhibits ASFV replication in vitro, and we have

successfully engineered pig strains to express this anti-ASFV CRISPR-Cas system constitutively. Despite not observing survival advantages in these transgenic pigs upon ASFV challenges, we did note a delay in infection in some cases. To the best of our knowledge, this study constitutes the first example of a germline-edited animal with an anti-virus CRISPR-Cas system. These findings contribute to the advancement of future anti-viral strategies and the optimization of viral immunity technologies.

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Keywords: African swine fever virus; CRISPR; pig; agriculture; xenotransplantation

8. **Identification of host proteins that interact with African swine fever virus pE301R,** Menghan Shi, Niu Zhou, Mengchen Xiu, Xiangzhi Li, Fen Shan, Wu Chen, Wanping Li, Cheng-Ming Chiang, Xiaodong Wu, Youming Zhang, Aiying Li, Jingjing Cao,Engineering Microbiology,Volume 4, Issue 2,2024,100149,https://doi.org/10.1016/j.engmic.2024.100149.

Abstract:

African swine fever virus (ASFV) infection poses enormous threats and challenges to the global pig industry; however, no effective vaccine is available against ASFV, attributing to the huge viral genome (approximately189 kb) and numerous encoding products (>150 genes) due to the limited understanding on the molecular mechanisms of viral pathogenesis. Elucidating the host-factor/viral-protein interaction network will reveal new targets for developing novel antiviral therapies. Using proteomic analysis, we identified 255 cellular proteins that interact with the ASFV-encoded pE301R protein when transiently expressed in HEK293T cells. Gene ontology (GO) annotation, Kyoto Encyclopedia of Genes and Genomes (KEGG) database enrichment, and protein-protein interaction (PPI) network analyses revealed that pE301R-interacting host proteins are potentially involved in various biological processes, including protein translation and folding, response to stimulation, and mitochondrial transmembrane transport. The interactions of two putative cellular proteins (apoptosis inducing factor mitochondria associated 1 (AIFM1)

and vimentin (VIM)) with pE301R-apoptosis inducing factor have been verified by co-immunoprecipitation. Our study revealed the inhibitory role of pE301R in interferon (IFN) induction that involves VIM sequestration by pE301R, identified interactions between ASFV pE301R and cellular proteins, and predicted the potential function of pE301R and its associated biological processes, providing valuable information to enhance our understanding of viral protein function, pathogenesis, and potential candidates for the prevention and control of ASFV infection.

Keywords: African swine fever virus; pE301R protein; Protein-protein interaction network; GO and KEGG analysis; Interferon

9. **Identification and epitope mapping of anti-p72 single-chain antibody against African swine fever virus based on phage display antibody library,**Jin-xing SONG, Meng-xiang WANG, Yi-xuan ZHANG, Bo WAN, Yong-kun DU, Guo-qing ZHUANG, Zi-bin LI, Song-lin QIAO, Rui GENG, Ya-nan WU, Gai-ping ZHANG,Journal of Integrative Agriculture,Volume 22, Issue 9,2023,Pages 2834-2847,https://doi.org/10.1016/j.jia.2023.07.039.

Abstract:

African swine fever virus (ASFV) is a lethal pathogen that causes severe threats to the global swine industry and it has already had catastrophic socio-economic effects. To date, no licensed prophylactic vaccine exists. Limited knowledge exists about the major immunogens of ASFV and the epitope mapping of the key antigens. As such, there is a considerable requirement to understand the functional monoclonal antibodies (mAbs) and the epitope mapping may be of utmost importance in our understanding of immune responses and designing improved vaccines, therapeutics, and diagnostics. In this study, we generated an ASFV antibody phage-display library from ASFV convalescent swine PBMCs, further screened a specific ASFV major capsid protein (p72) single-chain antibody and fused with an IgG Fc fragment (scFv-83-Fc), which is a specific recognition antibody against ASFV Pig/HLJ/2018 strain. Using the scFv-83-Fc mAb, we selected a conserved epitope peptide (221MTGYKH226) of p72 retrieved from a phage-displayed random peptide library. Moreover, flow cytometry and cell uptake experiments demonstrated that the epitope peptide can significantly promote BMDCs maturation in vitro and could be effectively uptaken by DCs, which indicated its potential application in vaccine and diagnostic reagent development. Overall, this study provided a valuable platform for identifying targets for ASFV vaccine development, as well as to facilitate the optimization design of subunit vaccine and diagnostic reagents.

Keywords: phage display antibody library; single chain antibody (scFv); p72; epitope

10. **Comparative genomic and transcriptomic analyses of African swine fever virus strains**,Peng Lu, Jiaqiao Zhou, Sibo Wei, Konosuke Takada, Hayato Masutani, Suguru Okuda, Ken Okamoto, Michio Suzuki, Tomoya Kitamura, Kentaro Masujin, Takehiro Kokuho, Hideaki Itoh, Koji Nagata,Computational and Structural Biotechnology Journal,Volume 21,2023,Pages 4322-4335,https://doi.org/10.1016/j.csbj.2023.08.028.

Abstract:

African swine fever (ASF) is the most devastating disease caused by the African swine fever virus (ASFV), impacting the pig industry worldwide and threatening food security and biodiversity. Although two vaccines have been approved in Vietnam to combat ASFV, the complexity of the virus, with its numerous open reading frames (ORFs), necessitates a more diverse vaccine strategy. Therefore, we focused on identifying and investigating the potential vaccine targets for developing a broad-spectrum defense against the virus. This study collected the genomic and/or transcriptomic data of different ASFV strains, specifically from in vitro studies, focusing on comparisons between genotypes I, II, and X, from the National Center for Biotechnology Information (NCBI) database. The comprehensive analysis of the genomic and transcriptomic differences between high- and low-virulence strains revealed six early genes, 13 late genes, and six short genes as potentially essential ORFs associated with high-virulence. In addition, many other ORFs (e.g., 14 multigene family members) are worth investigating. The results of this study provided candidate ORFs for developing ASF vaccines and therapies.

Keywords: African swine fever; Open reading frames; Broad-spectrum defense

11. **African swine fever virus pB475L evades host antiviral innate immunity via targeting STAT2 to inhibit IFN-I signaling**,Zhao Huang, Zhanzhuo Mai, Cuiying Kong, Jianyi You, Sizhan Lin, Chenyang Gao, WenBo Zhang, Xiongnan Chen, Qingmei Xie, Heng Wang, Shengqiu Tang, Pei Zhou, Lang Gong, Guihong Zhang,Journal of Biological Chemistry,Volume 300, Issue 7,2024,107472,https://doi.org/10.1016/j.jbc.2024.107472.

Abstract:

African swine fever virus (ASFV) causes severe disease in domestic pigs and wild boars, seriously threatening the development of the global pig industry. Type I interferon (IFN-I) is an important component of innate immunity, inducing the transcription and expression of antiviral cytokines by activating Janus-activated kinase–signal transducer and activator of transcription (STAT). However, the underlying molecular mechanisms by which ASFV antagonizes IFN-I signaling have not been fully elucidated. Therefore, using coimmunoprecipitation, confocal microscopy, and dual luciferase reporter assay methods, we investigated these mechanisms and identified a novel

ASFV immunosuppressive protein, pB475L, which interacts with the C-terminal domain of STAT2. Consequently, pB475L inhibited IFN-I signaling by inhibiting STAT1 and STAT2 heterodimerization and nuclear translocation. Furthermore, we constructed an ASFV-B475L7PM mutant strain by homologous recombination, finding that ASFV-B475L7PM attenuated the inhibitory effects on IFN-I signaling compared to ASFV-WT. In summary, this study reveals a new mechanism by which ASFV impairs host innate immunity.

Keywords: African swine fever; pB475L; type I interferon; signal transducer and activator of transcription 2

12. **Theaflavin inhibits African swine fever virus replication by disrupting lipid metabolism through activation of the AMPK signaling pathway in virto,**Yang Chen, Zhi Wei, Zebu Song, Hao Chang, Yanchen Guo, Yankuo Sun, Heng Wang, Zezhong Zheng, Guihong Zhang,Virus Research,Volume 334,2023,199159,https://doi.org/10.1016/j.virusres.2023.199159.

Abstract:

African swine fever virus (ASFV) is the etiological agent of African swine fever (ASF), which is one of the most harmful swine diseases in the pig industry because of its nearly 100% mortality rate in domestic pigs and results in incalculable economic loss. Ever since ASF was initially reported, scientists have worked to develop anti-ASF vaccines; however, currently no clinically effective vaccine for ASF is available. Therefore, the development of novel measures to prevent ASFV infection and transmission is essential. In this study, we aimed to investigate the anti-ASF activity of theaflavin (TF), a natural compound mainly isolated from black tea. We found that TF potently inhibited ASFV replication at non-cytotoxic concentrations ex vivo in primary porcine alveolar macrophages (PAMs). Mechanistically, we found that TF inhibited ASFV replication by acting on cells rather than interacting directly with ASFV to inhibit viral replication. Further, we found that TF upregulated the AMPK (5'-AMP-activated protein kinase) signaling pathway in ASFV-infected and uninfected cells, and treatment with the AMPK agonist MK8722 upregulated the AMPK signaling pathway and inhibited ASFV proliferation in a dose-dependent manner. Notably, the effects of TF on AMPK activation and ASFV inhibition were partially reversed by the AMPK inhibitor dorsomorphin. In addition, we found that TF down-regulated the expression of genes related to lipid synthesis and decreased the intracellular accumulation of total cholesterol and total triglycerides in ASFV-infected cells, suggesting that TF may inhibit ASFV replication by disrupting lipid metabolism. In summary, our results demonstrated that TF is an ASFV infection inhibitor and revealed the mechanism by which ASFV replication is inhibited, providing a novel mechanism and potential lead compound for the development of anti-ASFV drugs.

Keywords: African swine fever virus; Theaflavin; AMPK signaling pathway; Lipid metabolism

13. **Quantitative risk assessment of African swine fever introduction into Spain by legal import of swine products,**Carolina Muñoz-Pérez, Beatriz Martínez-López, José Pablo Gómez-Vázquez, Cecilia Aguilar-Vega, Jaime Bosch, Satoshi Ito, Marta Martínez-Avilés, José Manuel Sánchez-Vizcaíno,Research in Veterinary Science,Volume 163,2023,104990,https://doi.org/10.1016/j.rvsc.2023.104990.

Abstract:

African swine fever (ASF) is currently threatening the global swine industry. Its unstoppable global spread poses a serious risk to Spain, one of the world's leading producers. Over the past years, there has been an increased global burden of ASF not only in swine but also swine products. Unfortunately, many pigs are not diagnosed before slaughter and their products are used for human consumption. These ASF-contaminated products are only a source for new ASF outbreaks when they are consumed by domestic pigs or wild boar, which may happen either by swill feeding or landfill access. This study presents a quantitative stochastic risk assessment model for the introduction of ASF into Spain via the legal import of swine products, specifically pork and pork products. Entry assessment, exposure assessment, consequence assessment and risk estimation were carried out. The results suggest an annual probability of ASF introduction into Spain of 1.74 × 10−4, the highest risk being represented by Hungary, Portugal, and Poland. Monthly risk distribution is homogeneously distributed throughout the year. Illegal trade and pork product movement for own consumption (e.g., air and ship passenger luggage) have not been taken into account due to the lack of available, accredited data sources. This limitation may have influenced the model's outcomes and, the risk of introduction might be higher than that estimated. Nevertheless, the results presented herein would contribute to allocating resources to areas at higher risk, improving prevention and control strategies and, ultimately, would help reduce the risk of ASF introduction into Spain.

Keywords: African swine fever; Introduction; Swine product; Import risk assessment; Spain

14. **Identification of a conserved G-quadruplex within the E165R of African swine fever virus (ASFV) as a potential antiviral target,**Wenhao Liu, Xinglin He, Yance Zhu, Yaqin Li, Zhihao Wang, Pengfei Li, Jiajia Pan, Jiang Wang, Beibei Chu, Guoyu Yang, Mengjia Zhang, Qigai He, Yongtao Li, Wentao Li, Chao Zhang,Journal of Biological Chemistry,Volume 300, Issue 7,2024,107453,https://doi.org/10.1016/j.jbc.2024.107453.

Abstract:

Identification of a conserved G-quadruplex in E165R of ASFVAfrican swine fever virus (ASFV) is a double-stranded DNA arbovirus with high transmissibility and mortality rates. It has caused immense economic losses to the global pig industry. Currently, no effective vaccines or medications are to combat ASFV infection. G-quadruplex (G4) structures have attracted increasing interest because of their regulatory role in vital biological processes. In this study, we identified a conserved G-rich sequence within the E165R gene of ASFV. Subsequently, using various methods, we verified that this sequence could fold into a parallel G4. In addition, the G4-stabilizers pyridostatin and 5,10,15,20-tetrakis-(N-methyl-4-pyridyl) porphin (TMPyP4) can bind and stabilize this G4 structure, thereby inhibiting E165R gene expression, and the inhibitory effect is associated with G4 formation. Moreover, the G4 ligand pyridostatin substantially impeded ASFV proliferation in Vero cells by reducing gene copy number and viral protein expression. These compelling findings suggest that G4 structures may represent a promising and novel antiviral target against ASFV.

Keywords: African swine fever virus; ASFV; G-quadruplex; G4 ligands; antiviral activity

15. **Modelling African swine fever introduction in diverse Australian feral pig populations,**Callum Shaw, Angus McLure, Kathryn Glass,Preventive Veterinary Medicine,Volume 228,2024,106212,https://doi.org/10.1016/j.prevetmed.2024.106212.

Abstract:

African swine fever (ASF) is a viral disease that affects domestic and feral pigs. While not currently present in Australia, ASF outbreaks have been reported nearby in Indonesia, Timor-Leste, and Papua New Guinea. Feral pigs are found in all Australian states and territories and are distributed in a variety of habitats. To investigate the impacts of an ASF introduction event in Australia, we used a stochastic network-based metapopulation feral pig model to simulate ASF outbreaks in different regions of Australia. Outbreak intensity and persistence in feral pig populations was governed by local pig recruitment rates, population size, carcass decay period, and, if applicable, metapopulation topology. In Northern Australia, the carcass decay period was too short for prolonged persistence, while endemic transmission could possibly occur in cooler southern

areas. Populations in Macquarie Marshes in New South Wales and in Namadgi National Park in the Australian Capital Territory had the highest rates of persistence. The regions had different modes of transmission that led to long-term persistence. Endemic Macquarie Marshes simulations were characterised by rapid transmission caused by high population density that required a fragmented metapopulation to act as a bottleneck to slow transmission. Endemic simulations in Namadgi, with low density and relatively slow transmission, relied on large, well-connected populations coupled with long carcass decay times. Despite the potential for endemic transmission, both settings required potentially unlikely population sizes and dynamics for prolonged disease survival.

Keywords: African swine fever; Mathematical modelling; Feral pigs; Australia

16. **Multiepitope array as the key for African Swine Fever diagnosis,**Bruno Tilocca, Viviana Greco, Alessio Soggiu, Andrea Urbani, Domenico Britti, Luigi Bonizzi, Canio Buonavoglia, Paola Roncada,Veterinary Immunology and Immunopathology,Volume 257,2023,110548,https://doi.org/10.1016/j.vetimm.2023.110548.

Abstract:

African Swine Fever (ASF) is an acute hemorrhagic fever affecting suids with high mortality and morbidity rate. The causal agent of ASF, the African Swine Fever Virus (ASFV), is an icosahedral virus of 200 nm diameter, composed of an outer envelope layer of host derivation and a linear 170–190 kb long dsDNA molecule. As of today, no efficient therapeutic intervention nor prophylactic measures exist to fight ASFV diffusion, underlining the importance of the early diagnosis and the need for efficient in-field screening of ASF. Recommended guidelines for the diagnosis of ASF are unpracticable in the desirable context of the rapid in-farm screening. In this view, the design of innovative diagnostics based on a panel of multiple ASFV epitopes would amend versatility and the analytical performances of the deliverable, ensuring high quality and accuracy standards worth of implementation in rapid in-field monitoring programs. Pursuing this view, we performed epitope prediction from the major AFSV structural proteins holding the potential to be targeted in innovative rapid diagnostic tests. Selected ASFV structural protein sequences were retrieved from data repositories and their tridimensional structure was computed. Linear and 3D protein structures were subjected to the prediction of the epitope sequences, that are likely to elicit antibody production, by independent bioinformatic tools, providing a list of candidate biomarkers whose batch employment held the potential suitability for the unbiased rapid in-field diagnosis and, in turn, might be implemented in screening programs, crowing the current monitoring and control campaigns that are currently running worldwide.

Keywords: African swine fever virus; Multiepitope-based diagnosis; Bioinformatics; Immunoinformatics

17. **Thoughts on the research of African swine fever live-attenuated vaccines,** Xuefei Chu, Shengqiang Ge, Yuanyuan Zuo, Jin Cui, Zhou Sha, Naijun Han, Bingrong Wu, Bo Ni, Hui Zhang, Yan Lv, Zhiliang Wang, Yihong Xiao,Vaccine,Volume 42, Issue 25,2024,126052,https://doi.org/10.1016/j.vaccine.2024.06.020.

Abstract:

African swine fever (ASF) is a contagious and fatal disease caused by the African swine fever virus (ASFV), which can infect pigs of all breeds and ages. Most infected pigs have poor prognosis, leading to substantial economic losses for the global pig industry. Therefore, it is imperative to develop a safe and efficient commercial vaccine against ASF. The development of ASF vaccine can be traced back to 1960. However, because of its large genome, numerous encoded proteins, and complex virus particle structure, currently, no effective commercial vaccine is available. Several strategies have been applied in vaccine design, some of which are potential candidates for vaccine development. This review provides a comprehensive analysis on the safety and effectiveness, suboptimal immunization effects at high doses, absence of standardized evaluation criteria, notable variations among strains of the same genotype, and the substantial impact of animal health on the protective efficacy against viral challenge. All the information will be helpful to the ASF vaccine development.

Keywords: Live attenuated vaccine; Gene-deficient vaccine strain; Immunization; Challenge protection; Safty

18. **African swine fever virus early protein pI73R suppresses the type-I IFN promoter activities,** Danh Cong Lai, Jayeshbhai Chaudhari, Hiep L.X. Vu,Virus Research,Volume 343,2024,199342,https://doi.org/10.1016/j.virusres.2024.199342.

Abstract:

African swine fever virus is known to suppress type-I interferon (IFN) responses. The main objective of this study was to screen early-expressed viral genes for their ability to suppress IFN production. Out of 16 early genes examined, I73R exhibited robust suppression of cGAS-STING-induced IFN-β promoter activities, impeding the function of both IRF3 and NF-κB transcription factors. As a result, I73R obstructed IRF3 nuclear translocation following the treatment of cells with poly(dA:dT), a strong inducer of the cGAS-STING signaling pathway. Although the I73R

protein exhibits structural homology with the Zα domain binding to the left-handed helical form of DNA known as Z-DNA, its ability to suppress cGAS-STING induction of IFN-β was independent of Z-DNA binding activity. Instead, the α3 and β1 domains of I73R played a significant role in suppressing cGAS-STING induction of IFN-β. These findings offer insights into the protein's functions and support its role as a virulence factor.

Keywords: cGAS-STING signaling; Interferon suppression; Viral virulence gene; Z-DNA binding protein

19. **Nanobodies against African swine fever virus p72 and CD2v proteins as reagents for developing two cELISAs to detect viral antibodies,** Jiahong Zhu, Qingyuan Liu, Liuya Li, Runyu Zhang, Yueting Chang, Jiakai Zhao, Siyu Liu, Xinyu Zhao, Xu Chen, Yani Sun, Qin Zhao,Virologica Sinica,Volume 39, Issue 3,2024,Pages 478-489,https://doi.org/10.1016/j.virs.2024.04.002.

Abstract:

African swine fever virus (ASFV) poses a significant threat to the global swine industry. Currently, there are no effective vaccines or treatments available to combat ASFV infection in pigs. The primary means of controlling the spread of the disease is through rapid detection and subsequent elimination of infected pig. Recently, a lower virulent ASFV isolate with a deleted EP402R gene (CD2v-deleted) has been reported in China, which further complicates the control of ASFV infection in pig farms. Furthermore, an EP402R-deleted ASFV variant has been developed as a potential live attenuated vaccine candidate strain. Therefore, it is crucial to develop detection methods that can distinguish wild-type and EP402R-deleted ASFV infections. In this study, two recombinant ASFV-p72 and -CD2v proteins were expressed using a prokaryotic system and used to immunize Bactrian camels. Subsequently, eight nanobodies against ASFV-p72 and ten nanobodies against ASFV-CD2v were screened. Following the production of these nanobodies with horse radish peroxidase (HRP) fusion proteins, the ASFV-p72-Nb2-HRP and ASFV-CD2v-Nb22-HRP fusions were selected for the development of two competitive ELISAs (cELISAs) to detect anti-ASFV antibodies. The two cELISAs exhibited high sensitivity, good specificity, repeatability, and stability. The coincidence rate between the two cELISAs and commercial ELISA kits was 98.6% and 97.6%, respectively. Collectively, the two cELISA for detecting antibodies against ASFV demonstrated ease of operation, a low cost, and a simple production process. The two cELISAs could determine whether pigs were infected with wild-type or CD2v-deleted ASFV, and could play an important role in monitoring ASFV infections in pig farms.

Keywords: African swine fever virus (ASFV); ASFV-p72; ASFV-CD2v; Nanobody-HRP; Competitive ELISA

20. **Immune responses induced by a recombinant C-strain of classical swine fever virus expressing the F317L protein of African swine fever virus,** Shuwen Li, Yuxuan Gao, Huanjie Zhai, Xiangyu Guan, Xiaoke Yang, Qinghe Hou, Xinyu Zhang, Lian-Feng Li, Xiao Wang, Shujian Huang, Hua-Ji Qiu, Yongfeng Li,Veterinary Microbiology,Volume 298,2024,110239,https://doi.org/10.1016/j.vetmic.2024.110239.

Abstract:

African swine fever (ASF), a highly infectious and devastating disease affecting both domestic pigs and wild boars, owes its etiology to African swine fever virus (ASFV). ASFV encodes more than 165 proteins. However, novel immunogenic proteins remain unknown. This study aimed to determine the antigenicity of the F317L protein (pF317L) of ASFV. The results revealed that pF317L was able to react with convalescent pig sera, indicating that pF317L could be a candidate antigen. The antigenic potential of pF317L expressed by rHCLV-F317L, a recombinant virus in the backbone of C-strain (a lapinized live attenuated classical swine fever virus) was further investigated in rabbits and pigs. The results revealed that antibodies and cell-mediated immune responses against pF317L were induced in either rabbits or pigs inoculated with rHCLV-F317L. Importantly, anti-pF317L antibodies from rabbits or pigs immunized with rHCLV-F317L significantly inhibited ASFV replication in vitro. In conclusion, pF317L demonstrates favorable immunogenic properties, positioning it as a promising candidate for the development of protective antigens in the ongoing endeavor to formulate efficacious ASF vaccine strategies.

Keywords: African swine fever virus; F317L protein; Antigenicity; Cell-mediated immune responses

21. **African swine fever virus A137R protein inhibits NF-κB activation via suppression of MyD88 signaling in PK15 and 3D4/21 cells in vitro,** Yang Xu, Lei Wu, Jinxuan Hong, Xiaojuan Chi, Meichun Zheng, Liwei Wang, Ji-Long Chen, Guijie Guo,Veterinary Microbiology,Volume 292,2024,110067,https://doi.org/10.1016/j.vetmic.2024.110067.

Abstract:

African swine fever (ASF) is an infectious disease with high mortality caused by African swine fever virus (ASFV), which poses a great threat to the global swine industry. ASFV has evolved multiple strategies to evade host antiviral innate immunity by perturbing inflammatory responses and interferon production. However, the molecular mechanisms underlying manipulation of inflammatory responses by ASFV proteins are not fully understood. Here, we report that A137R protein of ASFV is a key suppressor of host inflammatory responses. Ectopic expression of ASFV A137R in HEK293T cells significantly inhibited the activation of IL-8 and NF-κB

promoters triggered by Sendai virus (SeV), influenza A virus (IAV), or vesicular stomatitis virus (VSV). Accordingly, forced A137R expression caused a significant decrease in the production of several inflammatory cytokines such as IL-8, IL-6 and TNF-α in the cells infected with SeV or IAV. Similar results were obtained from experiments using A137R overexpressing PK15 and 3D4/21 cells infected with SeV or VSV. Furthermore, we observed that A137R impaired the activation of MAPK and NF-κB signaling pathways, as enhanced expression of A137R significantly decreased the phosphorylation of JNK, p38 and p65 respectively upon viral infection (SeV or IAV) and IL-1β treatment. Mechanistically, we found that A137R interacted with MyD88, and dampened MyD88-mediated activation of MAPK and NF-κB signaling. Together, these findings uncover a critical role of A137R in restraining host inflammatory responses, and improve our understanding of complicated mechanisms whereby ASFV evades innate immunity.

Keywords: ASFV; A137R; NF-κB; Inflammatory response; MyD88

22. **Advancement in the development of gene/protein-based vaccines against African swine fever virus**,Ning Wang, Pan Huang, Jun Zhang, Minqi Lin, Xiaoru Lai, Jianwen Chen, Chungen Pan,Current Research in Microbial Sciences,Volume 6,2024,100232,https://doi.org/10.1016/j.crmicr.2024.100232.

Abstract:

African swine fever (ASF) is a highly contagious acute hemorrhagic viral disease, with the mortality rate of up to 100 % in domestic pigs. In recent years, ASF outbreaks have caused huge economic losses in numerous countries and regions, especially in Asia. Therefore, there is a pressing need to develop safe and effective vaccines against infection of the causative pathogen, African swine fever virus (ASFV). ASFV contains a large genome composed of double-stranded DNA with a size of 170–194 kb, which encodes nearly 200 viral proteins. Understanding the function of these complex genes/proteins and their roles in the generation of protective immunity will help in the development of ASFV vaccines. In this article, the gene/protein-based vaccine candidate are summarized, and the structural proteins which have been previously reported to protect animals from the virus challenge were emphatically described.

Keywords: African swine fever virus; Vaccine; Immunization regimen; Structural proteins; Specific immunity

23. **On-site detection and differentiation of African swine fever virus variants using an orthogonal CRISPR-Cas12b/Cas13a-based assay,**Zhe Wang, Yu Wang, Ying Zhang, Guosong Qin, Wenbo Sun, Aiping Wang, Yanfang Wang, Gaiping Zhang, Jianguo Zhao,iScience,Volume 27, Issue 4,2024,109050,https://doi.org/10.1016/j.isci.2024.109050.

Summary

The African swine fever virus (ASFV) and its variants have induced substantial economic losses in China, prompting a critical need for efficient detection methods. Several PCR-based methods have been developed to discriminate between wild-type ASFV and gene-deleted variants. However, the requirement for sophisticated equipment and skilled operators limits their use in field settings. Here, we developed a CRISPR-Cas12b/Cas13a-based detection assay that can identify ASFV variants with minimal equipment requirements and a short turnaround time. The assay utilizes the distinct DNA/RNA collateral cleavage preferences of Cas12b/Cas13a to detect two amplified targets from multiplex recombinase polymerase amplification (RPA) in a single tube, and the results can be visualized through fluorescent or lateral-flow readouts. When tested with clinical samples in field settings, our assay successfully detected all ASFV-positive samples in less than 60 min. This assay provides a rapid on-site surveillance tool for detecting ASFV and its emerging variants.

Keywords: Virology

24. **African swine fever virus MGF-360-10L is a novel and crucial virulence factor that mediates ubiquitination and degradation of JAK1 by recruiting the E3 ubiquitin ligase HERC5,**Dan Li, Jiangling Peng, Junhuang Wu, Jiamin Yi, Panxue Wu, Xiaolan Qi, Jingjing Ren, Gaochuang Peng, Xianghan Duan, Yi Ru, Huanan Liu, Hong Tian, Haixue Zheng, Chunfu Zheng,mBio,Volume 14, Issue 4,2023,https://doi.org/10.1128/mbio.00606-23.

Abstract:

African swine fever virus (ASFV) causes acute hemorrhagic infectious disease in pigs. The ASFV genome encodes various proteins that enable the virus to escape innate immunity; however, the underlying mechanisms are poorly understood. The present study found that ASFV MGF-360-10L significantly inhibits interferon (IFN)-β-triggered STAT1/2 promoter activation and the production of downstream IFN-stimulated genes (ISGs). ASFV MGF-360-10L deletion (ASFV-Δ10L) replication was impaired compared with the parental ASFV CN/GS/2018 strain, and more ISGs were induced by the ASFV-Δ10L in porcine alveolar macrophages in vitro. We found that MGF-360-10L mainly targets JAK1 and mediates its degradation in a dose-dependent manner.

Meanwhile, MGF-360-10L also mediates the K48-linked ubiquitination of JAK1 at lysine residues 245 and 269 by recruiting the E3 ubiquitin ligase HERC5 (HECT and RLD domain-containing E3 ubiquitin protein ligase 5). The virulence of ASFV-Δ10L was significantly lower than that of the parental strain in vivo, which indicates that MGF-360-10L is a novel virulence factor of ASFV. Our findings elaborate the novel mechanism of MGF-360-10L on the STAT1/2 signaling pathway, expanding our understanding of the inhibition of host innate immunity by ASFV-encoded proteins and providing novel insights that could contribute to the development of African swine fever vaccines.

IMPORTANCE

African swine fever outbreaks remain a concern in some areas. There is no effective drug or commercial vaccine to prevent African swine fever virus (ASFV) infection. In the present study, we found that overexpression of MGF-360-10L strongly inhibited the interferon (IFN)-β-induced STAT1/2 signaling pathway and the production of IFN-stimulated genes (ISGs). Furthermore, we demonstrated that MGF-360-10L mediates the degradation and K48-linked ubiquitination of JAK1 by recruiting the E3 ubiquitin ligase HERC5. The virulence of ASFV with MGF-360-10L deletion was significantly less than parental ASFV CN/GS/2018. Our study identified a new virulence factor and revealed a novel mechanism by which MGF-360-10L inhibits the immune response, thus providing new insights into the vaccination strategies against ASFV.

African swine fever outbreaks remain a concern in some areas. There is no effective drug or commercial vaccine to prevent African swine fever virus (ASFV) infection. In the present study, we found that overexpression of MGF-360-10L strongly inhibited the interferon (IFN)-β-induced STAT1/2 signaling pathway and the production of IFN-stimulated genes (ISGs). Furthermore, we demonstrated that MGF-360-10L mediates the degradation and K48-linked ubiquitination of JAK1 by recruiting the E3 ubiquitin ligase HERC5. The virulence of ASFV with MGF-360-10L deletion was significantly less than parental ASFV CN/GS/2018. Our study identified a new virulence factor and revealed a novel mechanism by which MGF-360-10L inhibits the immune response, thus providing new insights into the vaccination strategies against ASFV.

Keywords: African swine fever virus; MGF-360-10L; HERC5; JAK1; ubiquitination

25. **The evolutionary and genetic patterns of African swine fever virus,** Myeongji Cho, Xianglan Min, Nara Been, Hyeon S. Son,Infection, Genetics and Evolution,Volume 122,2024,105612,https://doi.org/10.1016/j.meegid.2024.105612.

Abstract:

African swine fever (ASF) is a serious animal disease, and has spread to Africa, Europe and Asia, causing massive economic losses. African swine fever virus (ASFV) is transmitted from a reservoir host (warthog) to domestic pigs via a sylvatic cycle (transmission between warthogs and soft ticks) and a domestic cycle (transmission between domestic pigs) and survives by expressing a variety of genes related to virus–host interactions. We evaluated differences in codon usage patterns among ASFV genotypes and clades and explored the common and specific evolutionary and genetic characteristics of ASFV sequences. We analysed the evolutionary relationships, nucleotide compositions, codon usage patterns, selection pressures (mutational pressure and natural selection) and viral adaptation to host codon usage based on the coding sequences (CDS) of key functional genes of ASFV. AT bias was detected in the six genes analysed, irrespective of clade. The AT bias of genes (A224L, A179L, EP153R) encoding proteins involved in interaction with host cells after infection was high; among them, the AT bias of EP153R was the greatest at 78.3%. A large number of overrepresented codons were identified in EP153R, whereas there were no overrepresented codons with a relative synonymous codon usage (RSCU) value of ≥3 in B646L. In most genes, the pattern of selection pressure was similar for each clade, but in EP153R, diverse patterns of selection pressure were captured within the same clade and genotype. As a result of evaluating host adaptation based on the codon adaptation index (CAI), for B646L, E183L, CP204L and A179L, the codon usage patterns in all sequences were more similar to tick than domestic pig or wild boar. However, EP153R showed the lowest average CAI value of 0.52 when selecting tick as a reference set. The genes analysed in this study showed different magnitudes of selection pressure at the clade and genotype levels, which is likely to be related to the function of the encoded proteins and may determine key evolutionary traits of viruses, such as the level of genetic variation and host range. The diversity of codon adaptations at the genetic level in ASFV may account for differences in translational selection in ASFV hosts and provides insight into viral host adaptation and co-evolution.

Keywords: ASFV; Codon usage pattern; RSCU; Mutational pressure; Natural selection

26. **Innate immune escape and adaptive immune evasion of African swine fever virus: A review,** Sai Niu, Yilin Guo, Xueying Wang, Zixuan Wang, Limeng Sun, Hanchuan Dai, Guiqing Peng,Virology,Volume 587,2023,109878,https://doi.org/10.1016/j.virol.2023.109878.

Abstract:

African swine fever virus (ASFV) causes hemorrhagic fever in domestic and wild pigs. The continued spread of the virus in Africa, Europe and Asia threatens the global pig industry. The lack of an effective vaccine limits disease control. ASFV has evolved a variety of encoded immune escape proteins and can evade host adaptive immunity, inducing cellular inflammation, autophagy, or apoptosis in host cells. Frequent persistent infections hinder the development of a viral vaccine and impose technical barriers. Currently, knowledge of the virulence-related genes, main pathogenic genes and immunoregulatory mechanism of ASFV is not comprehensive. We explain that ASFV invades the host to regulate its inflammatory response, interferon production, antigen presentation and cellular immunity. Furthermore, we propose potential ideas for ASFV vaccine target design, such as knocking out high-virulence genes in ASFV and performing data mining to identify the main genes that induce antiviral responses. To support a rational strategy for vaccine development, a better understanding of how ASFV interacts with the host and regulates the host's response to infection is needed. We review the current knowledge about ASFV targeting of host innate and adaptive immunity and the mechanisms by which the affected immune pathways are suppressed.

Keywords: ASFV; Innate immune; Adaptive immune; Escape; Inhibition

27. **African Swine Fever Virus pF778R Attenuates Type I Interferon Response by Impeding STAT1 Nuclear Translocation,** Qichao Chen, Liang Li, Lixinjie Liu, Zhankui Liu, Shibang Guo, Chen Tan, Huanchun Chen, Xiangru Wang,Virus Research,Volume 335,2023,199190,https://doi.org/10.1016/j.virusres.2023.199190.

Abstract:

African swine fever virus (ASFV) is an extensive and intricate double-stranded DNA virus with approximately 100% lethality in domestic swine. There is no effective vaccine to combat this virus, and this has led to substantial economic losses in the swine industry. ASFV encodes various proteins that impede interferon-based immune defenses in the host by employing diverse mechanisms. However, the roles of most of these proteins remain unknown. Therefore, understanding the immune evasion mechanisms employed by ASFV may facilitate the development of effective measures against the virus. In this study, we discovered a negative

regulation of the type I interferon (IFN) response by the ASFV ribonuclease reductase large subunit pF778R. This novel type Ⅰ IFN response antagonist significantly inhibits IFN-α-induced interferon-stimulated response element promoter activation, precludes the upregulation of various interferon-stimulated genes, and prevents STAT1 nuclear translocation. Mechanistically, pF778R did not affect the protein levels of crucial molecules in the JAK/STAT signaling pathway or engage in direct interactions. However, pF778R expression impedes type I IFN responses mediated by the JAK/STAT signaling pathway. Further investigations revealed that pF778R did not interfere with STAT1 phosphorylation or dimerization, but it inhibited IFN signaling by weakening the nuclear accumulation of activated STAT1. The critical role of the ASFV protein pF778R in evading IFN-I-mediated innate immunity highlights a unique mode of ASFV evasion and provides insights into the pathogenic mechanism of the virus.

Keywords: African swine fever virus; pF778R; Type I IFN signaling; STAT1 nuclear translocation; Immune evasion

28. **African swine fever virus MGF360-9L promotes viral replication by degrading the host protein HAX1,** Jinke Yang, Bo Yang, Yu Hao, Xijuan Shi, Xing Yang, Dajun Zhang, Dengshuai Zhao, Wenqian Yan, Lingling Chen, Xintian Bie, Guohui Chen, Zixiang Zhu, Dan Li, Chaochao Shen, Guoli Li, Xiangtao Liu, Haixue Zheng, Keshan Zhang,Virus Research,Volume 336,2023,199198,https://doi.org/10.1016/j.virusres.2023.199198.

Abstract:

African swine fever virus (ASFV) infection causes African swine fever (ASF), a virulent infectious disease that threatens the safety of livestock worldwide. Studies have shown that MGF360–9 L is important for the virulence of ASFV and the host protein HS1-associated protein X-1 (HAX1) plays an important role in viral pathogenesis. This study aimed to clarify the mechanism by which HAX1 mediates ASFV replication through interactions with MGF360–9 L. The regions of interaction between MGF360–9 L and HAX1 were predicted and validated. HAX1 overexpression and RNA interference studies revealed that HAX1 is a host restriction factor that suppresses ASFV replication. Moreover, HAX1 expression was inhibited in ASFV-infected mature bone marrow–derived macrophages, and infection with the virulent MGF360–9 L gene deletion strain (ΔMGF360–9 L) attenuated the inhibitory effect of the wild-type strain (WT) on HAX1 expression, suggesting a complex regulatory relationship between MGF360–9 L and HAX1. Furthermore, the E3 ubiquitin ligase RNF114 interacted with MGF360–9 L and HAX1, MGF360–9 L degraded HAX1 through the ubiquitin–proteasome pathway, and RNF114 facilitated the degradation of HAX1 by MGF360–9L-linked K48 ubiquitin chains through the ubiquitin–proteasome pathway, thereby facilitating ASFV replication. In conclusion, this study has enriched our understanding of the

regulatory networks between ASFV proteins and host proteins and provided a reference for investigation into the pathogenesis and immune escape mechanism of ASFV.

Keywords: ASFV; MGF360-9 L; HAX1; Protein interactions; Apoptosis; Virus replication

29. **Identification of two novel B cell epitopes on E184L protein of African swine fever virus using monoclonal antibodies,**Weldu Tesfagaber, Desong Lan, Wan Wang, Rui Zhao, Li Yin, Mingyang Yang, Yuanmao Zhu, Encheng Sun, Renqiang Liu, Wenjun Lin, Zhigao Bu, Fang Li, Dongming Zhao,Virus Research,Volume 346,2024,199412,https://doi.org/10.1016/j.virusres.2024.199412.

Abstract:

African swine fever virus (ASFV) is a large double-stranded DNA virus with a complex structural architecture and encodes more than 150 proteins, where many are with unknown functions. E184L has been reported as one of the immunogenic ASFV proteins that may contribute to ASFV pathogenesis and immune evasion. However, the antigenic epitopes of E184L are not yet characterized. In this study, recombinant E184L protein was expressed in prokaryotic expression system and four monoclonal antibodies (mAbs), designated as 1A10, 2D2, 3H6, and 4C10 were generated. All four mAbs reacted specifically with ASFV infected cells. To identify the epitopes of the mAbs, a series of overlapped peptides of E184L were designed and expressed as maltose binding fusion proteins. Accordingly, the expressed fusion proteins were probed with each E184L mAb separately by using Western blot. Following a fine mapping, the minimal linear epitope recognized by mAb 1A10 was identified as 119IQRQGFL125, and mAbs 2D2, 3H6, and 4C10 recognized a region located between 153DPTEFF158. Alignment of amino acids of E184L revealed that the two linear epitopes are highly conserved among different ASFV isolates. Furthermore, the potential application of the two epitopes in ASFV diagnosis was assessed through epitope-based ELISA using 24 ASFV positive and 18 negative pig serum and the method were able to distinguish positive and negative samples, indicating the two epitopes are dominant antigenic sites. To our knowledge, this is the first study to characterize the B cell epitopes of the antigenic E184L protein of ASFV, offering valuable tools for future research, as well as laying a foundation for serological diagnosis and epitope-based marker vaccine development.

Keywords: African swine fever virus; E184L; Linear B cell epitope; Monoclonal antibody

30. **Deletion of the B125R gene in the African swine fever virus SY18 strain leads to an A104R frameshift mutation slightly attenuating virulence in domestic pigs,** Rongnian Zhu, Ying Wang, Han Zhang, Jinjin Yang, Jiaqi Fan, Yanyan Zhang, Yu Wang, Qixuan Li, Xintao Zhou, Huixian Yue, Yu Qi, Shuchao Wang, Teng Chen, Shoufeng Zhang, Rongliang Hu,Virus Research,Volume 343,2024,199343,https://doi.org/10.1016/j.virusres.2024.199343.

Abstract:

African swine fever (ASF), caused by the ASF virus (ASFV), is a hemorrhagic and fatal viral disease that affects Eurasian wild boars and domestic pigs, posing a substantial threat to the global pig breeding industry. ASFV, a double-stranded DNA virus, possesses a large genome containing up to 160 open reading frames, most of which exhibit unknown functions. The B125R gene of ASFV, located at the 105595–105972 bp site in the ASFV-SY18 genome, remains unexplored. In this study, we discovered that B125R deletion did not affect recombinant virus rescue, nor did it hinder viral replication during the intermediate growth phase. Although the virulence of the recombinant strain harboring this deletion was attenuated, intramuscular inoculation of the recombinant virus in pigs at doses of 102 or 104 TCID50 resulted in mortality. Moreover, sequencing analysis of six recombinant strains obtained from three independent experiments consistently revealed an adenine insertion at the 47367–47375 bp site in the A104R gene due to the B125R deletion, leading to premature termination of this gene. Intriguingly, this insertion did not influence the transcription of the A104R gene between the recombinant and parental strains. Consequently, we postulate that the deletion of the B125R gene in ASFV-SY18 or other genotype II strains may marginally attenuate virulence in domestic pigs.

Keywords: African swine fever virus; B125R deletion; A104R mutation; Virulence

31. **In silico identification of multi-target inhibitors from medicinal fungal metabolites against the base excision repair pathway proteins of African swine fever virus†† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4ra00819g,** Mark Andrian B. Macalalad, Fredmoore L. Orosco,RSC Advances,Volume 14, Issue 14,2024,Pages 10039-10055,https://doi.org/10.1039/d4ra00819g.

Abstract:

African swine fever virus (ASFV) has emerged as a serious threat to the pork industry resulting in significant economic losses and heightened concerns about food security. With no known cure presently available, existing control measures center on animal quarantine and culling.

Considering the severity and challenges posed by ASFV, it is imperative to discover new treatment strategies and implement additional measures to prevent its further spread. This study recognized the potential of 1830 fungal metabolites from medicinal fungi as antiviral compounds against base excision repair (BER) proteins of ASFV, specifically ASFVAP, ASFVPolX, and ASFVLig. A wide array of computer-aided drug discovery techniques were employed to carry out the virtual screening process: ADMET profiling revealed 319 molecules with excellent bioavailability and toxicity properties; consensus docking identified the 10 best-scoring ligands against all targets; molecular dynamics simulation elucidated the stability of the protein-ligand complexes; and MM/PB(GB)SA energy calculations predicted the binding energies of the compounds as well as the key residues integral to binding. Through in silico methods, we identified two theoretical lead candidates against ASFVAP, four against ASFVLig, and five against ASFVPolX. Two compounds, methyl ganoderate E and antcamphin M, exhibited potential multi-target inhibitory characteristics against ASFVPolX and ASFVLig, while compound cochlactone A showed promising antagonistic results against all three BER proteins. It is recommended to prioritize these hit compounds in future in vitro and in vivo studies to validate their potential as antiviral drugs against ASFV.

32. **An ultrasensitive strip sensor for rapid detection of African swine fever virus,** Mengjing ZHANG, Lingling GUO, Xinxin XU, Hua KUANG, Chuanlai XU, Liqiang LIU,Chinese Journal of Analytical Chemistry,Volume 52, Issue 7,2024,100416,https://doi.org/10.1016/j.cjac.2024.100416.

Abstract:

African swine fever (ASF) is a highly contagious disease caused by the African swine fever virus (ASFV) infecting pigs, which has caused huge economic losses in countries around the world. Currently, there is no effective vaccine, and the prevention and control of ASF is mainly through rapid detection, so it is particularly important to carry identify and develop rapid detection methods for ASFV. In this study, recombinant plasmid PET-28a(+)-p30 was constructed, and the recombinant protein was obtained by inducing expression and Ni+ resin affinity column purification. Mice were immunized with recombinant p30 protein, and after three immunizations, ten strains of hybridoma cells that stably secreted monoclonal antibodies (mAbs) against p30 protein were obtained by cell fusion and subcloning. A colloidal gold immunochromatography assay (GICA) based on double antibody sandwich technology was established to screen the paired antibodies, and the trapping and detecting antibodies were mAb-11F11 and mAb-7A8, respectively, with a detection limit of 1 ng/mL, which laid an important material foundation for the early detection of ASF in the future.

Keywords: African swine fever virus; Prokaryotic expression; Monoclonal antibody; Immunochromatography assay

33. **A sensitive luciferase reporter assay for the detection of infectious African swine fever virus,** Kemal Mehinagic, Matthias Liniger, Maksym Samoilenko, Nick Soltermann, Markus Gerber, Nicolas Ruggli,Journal of Virological Methods,Volume 323,2024,114854,https://doi.org/10.1016/j.jviromet.2023.114854.

Abstract:

African swine fever virus (ASFV) is a complex DNA virus causing severe hemorrhagic disease in domestic pigs and wild boar. The disease has spread worldwide, with important socio-economic consequences. Early virus detection and control measures are crucial as there are no effective vaccines nor antivirals on the market. While the diagnosis of ASFV is fast and based primarily on qPCR, the detection of infectious ASFV is a labor-intensive process requiring susceptible macrophages and subsequent antibody-based staining or hemadsorption. The latter cannot detect ASFV isolates devoid of functional CD2v (EP402R) expression. Here, we report the development of a plasmid-based reporter assay (RA) for the sensitive detection and titration of infectious ASFV. To this end, we constructed a plasmid for secreted NanoLuc luciferase (secNluc) expression driven by the ASFV DNA polymerase gene G1211R promoter. Infection of plasmid-transfected immortalized porcine kidney macrophages (IPKM) followed by measurement of secNluc from cell culture supernatants allowed reliable automated quantification of infectious ASFV. The RA-based titers matched the titers determined by conventional p72-staining or hemadsorption protocols. The novel assay is specific for ASFV as it does not detect classical swine fever virus nor porcine reproductive and respiratory syndrome virus. It is applicable to ASFV of different genotypes, virulence, and sources, including ASFV from sera and whole blood from infected pigs as well as non-hemadsorbing ASFV.

Keywords: ASFV; African swine fever virus; Live virus detection; Reporter assay; Virus titration; High-throughput screening

34. **African swine fever virus S273R protein antagonizes type I interferon production by interfering with TBK1 and IRF3 interaction**,Hui Li, Xiaojie Zheng, You Li, Yingqi Zhu, Yangyang Xu, Zilong Yu, Wen-Hai Feng,Virologica Sinica,Volume 38, Issue 6,2023,Pages 911-921,https://doi.org/10.1016/j.virs.2023.08.009.

Abstract:

African swine fever (ASF) is originally reported in East Africa as an acute hemorrhagic fever. African swine fever virus (ASFV) is a giant and complex DNA virus with icosahedral structure and encodes a variety of virulence factors to resist host innate immune response. S273R protein (pS273R), as a SUMO-1 specific cysteine protease, can affect viral packaging by cutting polymeric proteins. In this study, we found that pS273R was an important antagonistic viral factor that suppressed cGAS-STING-mediated type I interferon (IFN-I) production. A detailed analysis showed that pS273R inhibited IFN-I production by interacting with interferon regulatory factor 3 (IRF3). Subsequently, we showed that pS273R disrupted the association between TBK1 and IRF3, leading to the repressed IRF3 phosphorylation and dimerization. Deletion and point mutation analysis verified that pS273R impaired IFN-I production independent of its cysteine protease activity. These findings will help us further understand ASFV pathogenesis.

Keywords: African swine fever virus (ASFV); cGAS-STING; S273R; IRF3; TBK1; Type I interferon (IFN-I)

35. **Screening and identification of linear B-cell epitopes on structural proteins of African Swine Fever Virus**,Haiyan Lu, Junjun Shao, Wei Liu, Shandian Gao, Guangqing Zhou, Xiaoyu Ning, Haiyan Huang, Yijia Liu, Huiyun Chang,Virus Research,Volume 350,2024,199465,https://doi.org/10.1016/j.virusres.2024.199465.

Abstract:

This study aims to screen and identify linear B-cell epitopes on the structural proteins of African Swine Fever Virus (ASFV) to assist in the development of peptide-based vaccines. In experiments, 66 peptides of 12 structural proteins of ASFV were predicted as potential linear B-cell epitopes using bioinformatics tools and were designed; the potential epitope proteins carried the GST tag were expressed, purified, and subjected to antigenicity analysis with porcine antiserum against ASFV, and further identified based on their immunogenicity in mice. A total of 22 potential linear B-cell epitopes showed immunoreactivity and immunogenicity. Of these epitopes, 13 epitopes were firstly identified including 4 epitopes located in p72 (352–363, 416–434, 424–439, 496–530 aa), 3 epitopes located in pE248R (121–136, 138–169, 158–185 aa), and only one epitope of each protein of pH108R (33–46 aa), p17 (63–86 aa), pE120R (65–117 aa), pE199L (175–189 aa), p12

(36–56 aa) as well as pB438L (211–230 aa). Notably, the immunoreactivity of the epitopes from the 63-86 aa of p17 and the 65–117 aa of pE120R were the highest amongst identified epitopes, while the immunogenicity of epitopes from the 36–56 aa of p12, the 211–230 aa of pB438L, the 352–363 aa of p72 and the 63–86 aa of p17 were the best strong. The other 9 epitopes are partly overlapped with previous researches. These epitopes identified here will further enrich the database of ASFV epitope, as well as help to develop safe, effective epitope-based ASF vaccines and ASF diagnostic reagents.

Keywords: Screening; Linear b-cell epitopes; African Swine Fever Virus

36. **Tetrandrine (TET) inhibits African swine fever virus entry into cells by blocking the PI3K/Akt pathway,** Bingxu Qian, Yongxin Hu, Cong Liu, Dongxia Zheng, Xiuju Han, Mingxia Gong, Yanli Zou, Dexin Zeng, Kai Liao, Yurun Miao, Xiaodong Wu, Jianjun Dai, Zhiliang Wang, Feng Xue,Virus Research,Volume 339,2024,199258,https://doi.org/10.1016/j.virusres.2023.199258.

Abstract:

African Swine Fever Virus (ASFV) infection causes an acute and highly contagious disease in swine, resulting in significant economic losses and societal harm worldwide. Currently, there are no effective vaccines or antiviral drugs available for ASFV. Tetrandrine (TET) is extracted from the traditional Chinese herb Stephania tetrandrae, possesses diverse biological functions such as anti-inflammatory, anti-tumor, and antiviral activities. The study comprehensively evaluated the anti-ASFV effect of TET and validated it through biological assays. The dose-dependent inhibition of TET against ASFV was confirmed and a novel mechanism of TET's anti-ASFV activity was elucidated. TET effectively inhibits ASFV during internalization by blocking macropinocytosis through the inhibition of the PI3K/Akt pathway. The specific inhibitor LY294002, targeting the PI3K/Akt pathway, exhibits similar antiviral activity against ASFV as TET. Furthermore, the inhibitory effect of TET against other viruses such as Lumpy Skin Disease Virus (LSDV) and Porcine Epidemic Diarrhea Virus (PEDV) was also identified. Our findings suggest that TET effectively inhibits ASFV and reveal the potential for broad-spectrum antiviral drugs targeting the PI3K/Akt pathway.

Keywords: African swine fever virus; Tetrandrine; Macropinocytosis; PI3K/Akt; Broad-spectrum antiviral drug

37. **African swine fever virus pS273R antagonizes stress granule formation by cleaving the nucleating protein G3BP1 to facilitate viral replication**,Tingting Li, Xuewen Li, Xiao Wang, Xin Chen, Gaihong Zhao, Chuanxia Liu, Miaofei Bao, Jie Song, Jiangnan Li, Li Huang, Jun Rong, Kegong Tian, Junhua Deng, Jianzhong Zhu, Xuehui Cai, Zhigao Bu, Jun Zheng, Changjiang Weng,Journal of Biological Chemistry,Volume 299, Issue 7,2023,104844,https://doi.org/10.1016/j.jbc.2023.104844.

Abstract:

Cytoplasmic stress granules (SGs) are generally triggered by stress-induced translation arrest for storing mRNAs. Recently, it has been shown that SGs are regulated by different stimulators including viral infection, which is involved in the antiviral activity of host cells to limit viral propagation. To survive, several viruses have been reported to execute various strategies, such as modulating SG formation, to create optimal surroundings for viral replication. African swine fever virus (ASFV) is one of the most notorious pathogens in the global pig industry. However, the interplay between ASFV infection and SG formation remains largely unknown. In this study, we found that ASFV infection inhibited SG formation. Through SG inhibitory screening, we found that several ASFV-encoded proteins are involved in inhibition of SG formation. Among them, an ASFV S273R protein (pS273R), the only cysteine protease encoded by the ASFV genome, significantly affected SG formation. ASFV pS273R interacted with G3BP1 (Ras-GTPase-activating protein [SH3 domain] binding protein 1), a vital nucleating protein of SG formation. Furthermore, we found that ASFV pS273R cleaved G3BP1 at the G140–F141 to produce two fragments (G3BP1-N1–140 and G3BP1-C141–456). Interestingly, both the pS273R-cleaved fragments of G3BP1 lost the ability to induce SG formation and antiviral activity. Taken together, our finding reveals that the proteolytic cleavage of G3BP1 by ASFV pS273R is a novel mechanism by which ASFV counteracts host stress and innate antiviral responses.

Keywords: African swine fever virus; G3BP1; stress granules; IFN production; pS273R; viral replication

38. **Development of a fully automated chemiluminescent immunoassay for the quantitative and qualitative detection of antibodies against African swine fever virus p72,**Lei Wang, Duan Li, Daoping Zeng, Shuangyun Wang, Jianwen Wu, Yanlin Liu, Guoliang Peng, Zheng Xu, Hong Jia, Changxu Song,Microbiology Spectrum,Volume 12, Issue 10,2024,https://doi.org/10.1128/spectrum.00809-24.

Abstract:

African swine fever (ASF), caused by ASF virus (ASFV), is a highly infectious and severe hemorrhagic disease of pigs that causes major economic losses. Currently, no commercial vaccine is available and prevention and control of ASF relies mainly on early diagnosis. Here, a novel automated double antigen sandwich chemiluminescent immunoassay (DAgS-aCLIA) was developed to detect antibodies against ASFV p72 (p72-Ab). For this purpose, recombinant p72 trimer was produced, coupled to magnetic particles as carriers and labeled with acridinium ester as a signal trace. Finally, p72-Ab can be sensitively and rapidly measured on an automated chemiluminescent instrument. For quantitative analysis, a calibration curve was established with a laudable linearity range of 0.21 to 212.0 ng/mL (R2 = 0.9910) and a lower detection limit of 0.15 ng/mL. For qualitative analysis, a cut-off value was set at 1.50 ng/mL with a diagnostic sensitivity of 100.00% and specificity of 98.33%. Furthermore, antibody response to an ASF gene-deleted vaccine candidate can be accurately quantified using this DAgS-aCLIA, as evidenced by early seroconversion as early as 7 days post-immunization and high antibody levels. Compared with available enzyme-linked immunosorbent assays, this DAgS-aCLIA demonstrated a wider linearity range of 4 to 16-fold, and excellent analytical sensitivity and agreement of over 95.60%. In conclusion, our proposed DAgS-aCLIA would be an effective tool to support ASF epidemiological surveillance. IMPORTANCE African swine fever virus (ASFV) is highly contagious in wild boar and domestic pigs. There is currently no vaccine available for ASF, so serological testing is an important diagnostic tool. Traditional enzyme-linked immunosorbent assays provide only qualitative results and are time and resource consuming. This study will develop an automated chemiluminescent immunoassay (CLIA) that can quantitatively and qualitatively detect antibodies to ASFV p72, greatly reducing detection time and labour-intensive operation, and improving detection sensitivity and linearity range. This novel CLIA would serve as a reliable and convenient tool for ASF pandemic surveillance and vaccine development.

African swine fever virus (ASFV) is highly contagious in wild boar and domestic pigs. There is currently no vaccine available for ASF, so serological testing is an important diagnostic tool. Traditional enzyme-linked immunosorbent assays provide only qualitative results and are time and resource consuming. This study will develop an automated chemiluminescent immunoassay (CLIA) that can quantitatively and qualitatively detect antibodies to ASFV p72, greatly reducing detection time and labour-intensive operation, and improving detection sensitivity and linearity

range. This novel CLIA would serve as a reliable and convenient tool for ASF pandemic surveillance and vaccine development.

Keywords: ASFV p72; chemiluminescent immunoassay; antibody detection

39. **Identification of a novel linear B-cell epitope on the p30 protein of African swine fever virus using monoclonal antibodies,**Panpan Tian, Zhuoya Sun, Mengxiang Wang, Jinxing Song, Junru Sun, Lei Zhou, Dawei Jiang, Angke Zhang, Yanan Wu, Gaiping Zhang,Virus Research,Volume 341,2024,199328,https://doi.org/10.1016/j.virusres.2024.199328.

Abstract:

The outbreak of African Swine Fever (ASF) has caused huge economic losses to the pig industry. There are no safe and effective vaccines or diagnostics available. The p30 protein serves as a key target for the detection of ASFV antibodies and is an essential antigenic protein for early serological diagnosis. Here, the p30 protein was purified after being expressed in E. coli and its immunogenicity was verified in sera from pigs naturally infected with ASFV. Furthermore, a monoclonal antibody (McAb) designated as McAb 1B4G2–4 (subtype IgG1/kappa-type) was produced and it was verified to specifically recognize the ASFV Pig/HLJ/2018/strain and eukaryotic recombinant ASFV p30 protein. The epitope identified by McAb 1B4G2–4, defining the unique B-cell epitope 164HNFIQTI170, was located using peptide scanning. Comparing amino acid (aa) sequence revealed that this epitope is conserved in all reference ASFV strains from different regions of China, including the highly pathogenic strain Georgia 2007/1 (NC_044959.2) that is widely distributed. It is also exposed to the surface of the p30 protein, suggesting that it could be an important B-cell epitope. Our study may serve as a basis for the development of serological diagnostic methods and subunit vaccines.

Keywords: African swine fever; Vaccines; p30 protein; Monoclonal antibody; B-cell epitope

40. **Rhein suppresses African swine fever virus replication in vitro via activating the caspase-dependent mitochondrial apoptosis pathway,**Zebu Song, Yang Chen, Hao Chang, Yanchen Guo, Qi Gao, Zhi Wei, Lang Gong, Guihong Zhang, ZeZhong Zheng,Virus Research,Volume 338,2023,199238,https://doi.org/10.1016/j.virusres.2023.199238.

Abstract:

African swine fever (ASF) is a virulent infectious diseases of pigs caused by the African swine fever virus (ASFV) that can spread widely and cause high fatality rates. Currently, there is no effective

way to treat the disease, and there is no effective vaccine to prevent it. Rhein, an anthraquinone compound extracted from many traditional Chinese medicines, exhibits anti-inflammatory, anti-tumor, and anti-viral activities. However, the anti-viral effects of rhein on ASFV remain unclear. Therefore, this study aimed to investigate the anti-ASFV activity of rhein in porcine alveolar macrophages (PAMs) and the underlying mechanisms. In this study, we confirmed that rhein inhibits ASFV replication significantly in a dose-dependent manner in vitro. Moreover, rhein could alter the susceptibility of PAMs to ASFV and promoted the production of superoxide in the mitochondria, which induced the loss of mitochondrial membrane potential, leading to the activation of caspase-9, caspase-3, and apoptosis. Mito-TEMPO, a mitochondria-targeted antioxidant, blocked rhein-induced mitochondrial superoxide generation and loss of mitochondrial membrane potential, prevented caspase-9 and caspase-3 activation, alleviated apoptosis, and suppressed the anti-ASFV activity of rhein. Altogether, our results suggested that rhein could play an anti-ASFV role by inducing apoptosis through the activation of the caspase-dependent mitochondrial apoptotic pathway and may provide a novel compound for developing anti-ASFV drugs.

Keywords: Rhein; African swine fever virus; Antiviral; Mitochondrial apoptosis

41. **Development of high-concentration labeled colloidal gold immunochromatographic test strips for detecting african swine fever virus p30 protein antibodies,**Huai-cheng Liu, Rong-chao Liu, Mei-rong Hu, Ao-bing Yang, Ren-hu Wu, Yan Chen, Jin Zhang, Ji-shan Bai, Sheng-bo Wu, Jian-peng Chen, Yun-feng Long, Yan Jiang, Bin Zhou,Heliyon,Volume 10, Issue 3,2024,e25214,https://doi.org/10.1016/j.heliyon.2024.e25214.

Abstract:

African Swine Fever (ASF), caused by the African swine fever virus (ASFV), has inflicted significant economic losses on the pig industry in China. The key to mitigating its impact lies in accurate screening and strict biosecurity measures. In this regard, the development of colloidal gold immunochromatographic test strips (CGITS) has proven to be an effective method for detecting ASFV antibodies. These test strips are based on the ASFV p30 recombinant protein and corresponding monoclonal antibodies. The design of the test strip incorporates a high-concentration colloidal gold-labeled p30 recombinant protein as the detection sensor, utilizing Staphylococcal Protein A (SPA) as the test line (T line), and p30 monoclonal antibody as the control line (C line). The sensitivity and specificity of the test strip were evaluated after optimizing the labeling concentration, pH, and protein dosage. The research findings revealed that the optimal colloidal gold labeling concentration was 0.05 %, the optimal pH was 8.4, and the optimal protein dosage was 10 μg/mL. Under these conditions, the CGITS demonstrated a detection limit

of 1:512 dilution of ASFV standard positive serum, without exhibiting cross-reactivity with antibodies against other viral pathogens. Furthermore, the test strips remained stable for up to 20 days when stored at 50 °C and 4 °C. Comparatively, the CGITS outperformed commercial ELISA kits, displaying a sensitivity of 90.9 % and a specificity of 96.2 %. Subsequently, 108 clinical sera were tested to assess its performance. The data showed that the coincidence rate between the CGITS and ELISA was 93.5 %. In conclusion, the rapid colloidal gold test strip provides an efficient and reliable screening tool for on-site clinical detection of ASF in China. Its accuracy, stability, and simplicity make it a valuable asset in combating the spread of ASF and limiting its impact on the pig industry.

Keywords: African swine fever virus (ASFV); p30 protein; Monoclonal antibody; Colloidal gold immunochromatography; High-concentration labeling; Testing

42. **Targeted mutagenesis of the β-strand DNA binding region of African swine fever virus histone-like protein (pA104R) impairs DNA-binding activity and antibody recognition,**Ana Catarina Urbano, Nicolas Ferreira, Nuno Jordão, Fernando Boinas, Carlos Martins, Fernando Ferreira,Antiviral Research,Volume 221,2024,105784,https://doi.org/10.1016/j.antiviral.2023.105784.

Abstract:

African Swine Fever (ASF) is a highly contagious disease caused by a double-stranded DNA virus (ASFV). Despite significant advances made over the last decade, issues such as residual virulence and absence of differentiating infected from vaccinated animals (DIVA) capacity remain an obstacle in the development of live attenuated vaccines (LAVs) against ASFV. It is, therefore, necessary to identify novel strategies to improve vaccine safety, by rational mutagenesis of virulence associated genes and generation of DIVA markers. ASFV encodes a HU (histone-like protein from E. coli strain U93) homolog protein, pA104R, which is involved in viral genome assembly and host immune recognition. A phylogenetic analysis revealed that pA104R is highly conserved among ASFV isolates, suggesting that it can be a good target for vaccine design. Thus, we selectively mutated the β-strand DNA binding region (BDR) of pA104R to attenuate its enzymatic activity, and identified and mutated several B-cell epitopes present in pA104R to generate a negative marker. Residues K64, K66, and R69 in the BDR were identified as relevant for pA104R activity, with double mutation of the first two showing additive attenuation. pA104R-reactive IgM and IgG epitopes were also identified in the bottom of the BDR, with selective mutagenesis drastically reducing antibody recognition and, when combined with mutations in the arm of the BDR, leading to a further reduction of DNA-binding activity. Interestingly, the immunodominant pA104R-reactive IgG epitope was mainly recognized by IgG1 suggesting that

pA104R induces a dominant Th2 response. In sum, the rational mutagenesis can reduce pA104R-DNA binding activity and immune reactivity, providing a rationale for the development of an ASFV pA104R-based DIVA vaccine.

Keywords: ASFV; pA104R; Mutagenesis; Epitope screening; DIVA; Vaccine

43. **Benefits and costs of measures to tackle the outbreak of African swine fever in Sweden,** Ing-Marie Gren, Hans Andersson, Lars Jonasson,Preventive Veterinary Medicine,Volume 233,2024,106353,https://doi.org/10.1016/j.prevetmed.2024.106353.

Abstract:

A common rule in many countries for mitigating the damage caused by African swine fever (ASF) is to eradicate the virus at the outbreak in order to prevent its dispersal and the associated social costs of depopulating infected domestic pigs. The economic performance of this practice, as measured by five different evaluation criteria (net present value, benefit-cost ratio, rate of return, internal rate of return, and payback time), depends on the type of control cost and the spatial and dynamic allocation of benefits, i.e. avoided losses from infected domestic pig farms. The present paper calculates the direct and indirect costs of immediate control measures during an ASF outbreak in wild boars in Mid Sweden. The direct costs include expenses incurred for surveillance, laboratory tests, depopulation of wild boar etc., while the indirect costs are borne by firms and people in the area in relation to movement restrictions. The calculations showed that the total cost of control measures amounted to 28 million euros, with indirect costs making up 40 % of this figure. The benefits were greatly dependent on the speed of ASF dispersal and assumptions about pig farmers' investment responses, which implied large variations in each of the five evaluation criteria.

Keywords: Benefit-cost analysis; African swine fever; Direct and indirect costs; Virus spatial dispersal; Agriculture sector model; Sweden

44. **An integrated fuzzy multi-criteria decision-making model for determining the interdependencies among the african swine fever spread factors,**Hannah Jesse Lauron, Dharyll Prince Mariscal Abellana,Decision Analytics Journal,Volume 11,2024,100454,https://doi.org/10.1016/j.dajour.2024.100454.

Abstract:

African swine fever (ASF) is a disease outbreak that has substantially impacted agriculture, public health, and food security. This study tackles the lack of knowledge regarding the interactions between multiple factors causing the spread of ASF. We also present a potential for cross-fertilization between applying soft computing to nonmeasurable factors of different natures through investigating the relationships and significance of ASF spread factors in the Philippine setting using Multi-criteria Decision Making (MCDM) methodologies and fuzzy logic. We develop a list of ASF spread factors through a literature review. Fuzzy Analytic Hierarchy Process (AHP)-VIseKriterijumska Optimizacija I Kompromisno Resenje (VIKOR) is used for the dimensionality reduction of factors. These elements are then classified as either net causes or net effects using the Fuzzy Decision Making Trial and Evaluation Laboratory (DEMATEL), which also shows their interdependencies. The results show how socioeconomic characteristics, particularly those connected to biosecurity lapses, significantly influence other parameters through a causal graph. This knowledge can be used to develop effective ASF preventive and management methods as it provides a systematic and unbiased decision-making framework for policymakers, breeders, and manufacturers.

Keywords: Multi-criteria decision making; Soft computing; Analytic hierarchy process; VIKOR; DEMATEL; Fuzzy logic

45. **Epidemiological impacts of attenuated African swine fever virus circulating in wild boar populations,**Marta Martínez Avilés, Jaime Bosch, Benjamin Ivorra, Ángel Manuel Ramos, Satoshi Ito, José Ángel Barasona, José Manuel Sánchez-Vizcaíno,Research in Veterinary Science,Volume 162,2023,104964,https://doi.org/10.1016/j.rvsc.2023.104964.

Abstract:

African swine fever virus (ASFV) genotype II has been present in wild boar in the European Union since 2014. Control measures have reduced the incidence of the ASF, but highly virulent as well as attenuated ASFV strains continue to circulate. We present the intraherd epidemiological parameters of low and highly virulent ASFV in wild boar from experimental data, and for the first time, evaluate the impact of attenuated strain circulation through unique deterministic

compartmental model simulations under various potential scenarios and hypotheses. Using an estimated PCR infectious threshold of TPCR = 36.4, we obtained several transmission parameters, like an Rx (experimental intraherd R0) value of 4.5. We also introduce two novel epidemiological parameters: infectious power and resistance power, which indicate the ability of animals to transmit the infection and the reduction in infectiousness after successive exposures to varying virulence strains, respectively. The presence of ASFV attenuated strains results in 4–17% of animals either remaining in a carrier state or becoming susceptible again when exposed to highly virulent ASFV for more than two years. The timing between exposures to viruses of different virulence also influences the percentage of animals that die or remain susceptible. The findings of this study can be utilized in epidemiological modelling and provide insight into important risk situations that should be considered for surveillance and future potential ASF vaccination strategies in wild boar.

Keywords: African swine fever virus; Wild boar; Disease dynamics; ASF transmission; Modelling; Moderate or low virulence

46. **Immunogenicity and efficacy of an LNP-mRNA prepared from African Swine Fever Virus K205R1,**Chuanwen Tian, Yingnan Liu, Dongdong Di, Zhenhua Xie, Yao Li, Rongrong Wang, Jie Li, Jingyi Liu, Hongjun Chen,Journal of Integrative Agriculture,2024,https://doi.org/10.1016/j.jia.2024.03.053.

47. **Deletions of MGF110-9L and MGF360-9L from African swine fever virus are highly attenuated in swine and confer protection against homologous challenge,**Dan Li, Jingjing Ren, Guoqiang Zhu, Panxue Wu, Wenping Yang, Yi Ru, Tao Feng, Huanan Liu, Jing Zhang, Jiangling Peng, Hong Tian, Xiangtao Liu, Haixue Zheng,Journal of Biological Chemistry,Volume 299, Issue 6,2023,104767,https://doi.org/10.1016/j.jbc.2023.104767.

Abstract:

African swine fever, caused by a large icosahedral DNA virus (African swine fever virus, ASFV), is a highly contagious disease in domestic and feral swine, thus posing a significant economic threat to the global swine industry. Currently, there are no effective vaccines or the available methods to control ASFV infection. Attenuated live viruses with deleted virulence factors are considered to be the most promising vaccine candidates; however, the mechanism by which these attenuated viruses confer protection is unclear. Here, we used the Chinese ASFV CN/GS/2018 as a backbone and used homologous recombination to generate a virus in which MGF110-9L and MGF360-9L, two genes antagonize host innate antiviral immune response, were deleted (ASFV-

ΔMGF110/360-9L). This genetically modified virus was highly attenuated in pigs and provided effective protection of pigs against parental ASFV challenge. Importantly, we found ASFV-ΔMGF110/360-9L infection induced higher expression of Toll-like receptor 2 (TLR2) mRNA compared with parental ASFV as determined by RNA-Seq and RT-PCR analysis. Further immunoblotting results showed that parental ASFV and ASFV-ΔMGF110/360-9L infection inhibited Pam3CSK4-triggered activating phosphorylation of proinflammatory transcription factor NF-κB subunit p65 and phosphorylation of NF-κB inhibitor IκBα levels, although NF-κB activation was higher in ASFV-ΔMGF110/360-9L-infected cells compared with parental ASFV-infected cells. Additionally, we show overexpression of TLR2 inhibited ASFV replication and the expression of ASFV p72 protein, whereas knockdown of TLR2 had the opposite effect. Our findings suggest that the attenuated virulence of ASFV-ΔMGF110/360-9L might be mediated by increased NF-κB and TLR2 signaling.

Keywords: African swine fever virus; MGF110-9L; MGF360-9L; Toll-like receptor 2; RNA-seq

48. **Enhanced detection of African swine fever virus in samples with low viral load using digital PCR technology,** R. Yang, W.-G. Fu, J. Zhou, Y.-F. Zhang, L. Yang, H.-B. Yang, L.-Z. Fu,Heliyon,Volume 10, Issue 7,2024,e28426,https://doi.org/10.1016/j.heliyon.2024.e28426.

Abstract:

Detection of low viral load samples has long been a challenge for African swine fever (ASF) prevention and control. This study aimed to compare the detection efficacy of droplet digital PCR(ddPCR) and quantitative PCR(qPCR) for African swine fever virus (ASFV) at different viral loads, with a focus on assessing the accuracy of ddPCR in detecting low viral load samples. The results revealed that ddPCR had a detection limit of 1.97 (95% CI 1.48 – 4.12) copies/reaction and was 18.99 times more sensitive than qPCR (detection limit: 37.42, 95% CI 29.56 – 69.87 copies/reaction). In the quantification of high, medium, and low viral load samples, ddPCR showed superior stability with lower intra- (2.06% – 7.58%) and inter-assay (3.83% – 7.50%) coefficients of variation than those of qPCR (intra-assay: 8.08%–29.86%; inter-assay: 9.27%–34.58%). Bland-Altman analysis indicated acceptable consistency between ddPCR and qPCR for high and medium viral load samples; however, discrepancies were observed for low viral load samples, where two samples (2/24, 8.33%) exhibited deviations beyond the acceptable range (−46.18 copies/reaction). Moreover, ddPCR demonstrated better performance in detecting ASFV in clinical samples from asymptomatic pigs and environmental samples, with qPCR showing false negative rates of 7.69% (2/26) and 27.27% (12/44), respectively. McNemar analysis revealed significant differences between the two methods (P = 0.000) for samples with a viral load <100

copies/reaction. The results of this study demonstrate that ddPCR has better detection limits and adaptability than qPCR, allowing for a more accurate detection of ASFV in early-stage infections and low-concentration environmental samples. These findings highlight the potential of ddPCR in the prevention and control of ASF.

Keywords: African swine fever virus; Droplet digital PCR; Quantitative PCR

49. **Establishment of an indirect immunofluorescence assay for the detection of African swine fever virus antibodies**,Wan Wang, Zhenjiang Zhang, Weldu Tesfagaber, Jiwen Zhang, Fang Li, Encheng Sun, Lijie Tang, Zhigao Bu, Yuanmao Zhu, Dongming Zhao,Journal of Integrative Agriculture,Volume 23, Issue 1,2024,Pages 228-238,https://doi.org/10.1016/j.jia.2023.05.021.

Abstract:

African swine fever (ASF) continues to cause enormous economic loss to the global pig industry. Since there is no safe and effective vaccine, accurate and timely diagnosis of ASF is essential to implement control measures. Indirect immunofluorescence assay (IFA) is a gold standard serological method recommended by the World Organization for Animal Health (WOAH). In this study, we used primary fetal kidney cells to establish a wild boar cell line (BK2258) that supported the efficient replication of ASF virus (ASFV) SD/DY-I/21 and showed visible cytopathic effect (CPE). Moreover, using BK2258, we established a sensitive and specific IFA for ASFV antibody detection. To standardize and evaluate the performance of this assay, we used serum samples from pigs infected with the low virulent genotype I SD/DY-I/21 and genotype II HLJ/HRB1/20, and immunized with the vaccine candidate HLJ/18-7GD, field samples, and negative serum samples. The IFA reacted with the ASFV-positive sera and displayed bright fluorescence foci. There was no non-specific green fluorescence due to cellular senescence or other cell damage-causing factors. Compared to a commercial indirect enzyme-linked immunosorbent assay (iELISA), ASFV antibodies were detected 1–4 days earlier using our IFA. The detection limits of the IFA and iELISA for the same ASFV-antibody positive serum samples were 1:25,600 and 1:6,400, respectively, indicating that the IFA is more sensitive than iELISA. The newly established IFA was highly specific and did not cross-react with sera positive for six other important porcine pathogens (i.e., Classical swine fever virus (CSFV), Porcine reproductive and respiratory syndrome virus (PRRSV), Porcme circovirus type 2 (PCV2), Pseudorabies virus (PRV), Foot-and-Mouth disease virus type O (FMDV/O), and Porcine epidemic diarrhea virus (PEDV)). This study thus provides a sensitive, specific, and reliable detection method that is suitable for the serological diagnosis of ASF.

Keywords: African swine fever; antibody; IFA; serological method

50. **Comprehensive mapping of antigenic linear B-cell epitopes on K205R protein of African swine fever virus with monoclonal antibodies,** Shu-Jian Zhang, Jing Liu, Bei Niu, Yuan-Mao Zhu, Dong-Ming Zhao, Wei-Ye Chen, Ren-Qiang Liu, Zhi-Gao Bu, Rong-Hong Hua,Virus Research,Volume 328,2023,199085,https://doi.org/10.1016/j.virusres.2023.199085.

Abstract:

African swine fever virus causes an acute, highly contagious swine disease with high mortality, leading to enormous losses in the pig industry. The K205R, a nonstructural protein of African swine fever virus, is abundantly expressed in the cytoplasm of infected cells at the early stage of infection and induces a strong immune response. However, to date, the antigenic epitopes of this immunodeterminant have not been characterized. In the present study, the K205R protein was expressed in a mammalian cell line and purified using Ni-affinity chromatography. Furthermore, three monoclonal antibodies (mAbs; 5D6, 7A8, and 7H10) against K205R were generated. Indirect immunofluorescence assay and western blot results showed that all three mAbs recognized native and denatured K205R in African swine fever virus (ASFV)-infected cells. To identify the epitopes of the mAbs, a series of overlapping short peptides were designed and expressed as fusion proteins with maltose-binding protein. Subsequently, the peptide fusion proteins were probed with monoclonal antibodies using western blot and enzyme-linked immunosorbent assay. The three target epitopes were fine-mapped; the core sequences of recognized by the mAbs 5D6, 7A8, and 7H10 were identified as 157FLTPEIQAILDE168, 154REKFLTP160, and 136PTNAMFFTRSEWA148, respectively. Probing with sera from ASFV-infected pigs in a dot blot assay demonstrated that epitope 7H10 was the immunodominant epitope of K205R. Sequence alignment showed that all epitopes were conserved across ASFV strains and genotypes. To our knowledge, this is the first study to characterize the epitopes of the antigenic K205R protein of ASFV. These findings may serve as a basis for the development of serological diagnostic methods and subunit vaccines.

Keywords: African swine fever virus; K205R protein; Monoclonal antibody; B-cell epitope