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Review Article

Research Review on Non-structural Protein 3 (NS3) of Classical Swine Fever Virus and Its Potential Applications in Vaccine Development

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Abstract

Classical Swine Fever Virus (CSFV) is a virus that poses a serious threat to the pig farming industry, and its non-structural protein 3 (NS3) plays a key role in the virus's replication, pathogenicity, and immune evasion. In recent years, with the deepening research on CSFV NS3, its important roles in viral biology and immunology have gradually been revealed. NS3 is not only involved in the replication process of CSFV but also engages in complex interactions with the host immune system, promoting the virus's immune evasion. However, despite numerous studies exploring the functional mechanisms and structural characteristics of NS3, the specific applications of CSFV NS3 in vaccine development still face shortcomings and challenges. This article aims to review the latest research progress on CSFV NS3, analyze its potential as a vaccine target, and provide new ideas and directions for future vaccine development and virus control strategies.

Keywords: Classical Swine Fever Virus, CSFV, Immune evasion, Non-structural protein 3, NS3, Research progress, Vaccine development

Introduction

The Classical Swine Fever Virus (CSFV) is a highly contagious virus that primarily affects domestic pigs, leading to severe economic losses and the collapse of the pig farming industry. This virus belongs to the *Flaviviridae* family, and its transmission routes include direct contact, airborne spread, and indirect transmission through contaminated feed and equipment. Reports indicate that the prevalence of CSFV has not only caused significant economic losses to the pig farming industry but has also had a profound impact on the global pork supply chain [1,2]. In some countries, outbreaks have led to large-scale culling measures, further exacerbating the plight of the pig farming industry. Therefore, control measures against the swine fever virus are particularly important.

Non-structural protein 3 (NS3) is a key component in the CSFV life cycle, involved in the virus's replication and transcription processes. NS3 is not only one of the virus's main enzymes but also plays a crucial role in the interaction between the virus and host cells. Studies have shown that NS3 can suppress the host's immune response, helping the virus evade immune surveillance [3]. Furthermore, the functional and structural characteristics of NS3 make it a potential target for vaccine development and therapeutic interventions, making in-depth research on the biological properties of NS3 essential for understanding the pathogenic mechanisms of CSFV and developing effective control strategies.

The importance of studying NS3 is reflected not only in basic scientific research but also in providing new

insights for controlling swine fever outbreaks. Through in-depth research on NS3, scientists can reveal its specific roles in the virus's life cycle, thereby developing targeted interventions such as vaccines or antiviral drugs [4] *(Fig. 1)*. For example, vaccine development targeting NS3 may enhance the immune response in pigs, increasing their resistance to CSFV and thereby reducing the occurrence and spread of outbreaks. Therefore, NS3 is not only key to understanding the biology of CSFV but also an important target for formulating effective control strategies.

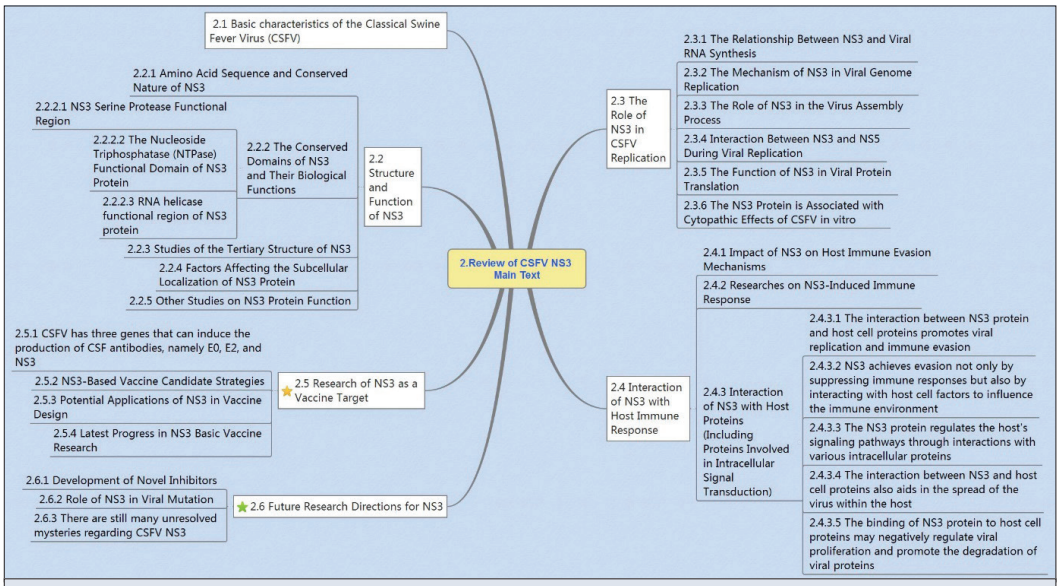

Fig 1. Study scheme on the review of NS3 protein of Classical Swine Fever Virus

Basic Characteristics of the CSFV

CSFV is an important animal virus belonging to the genus *Pestivirus* in the *Flaviviridae* family. Its genome is a positive-sense single-stranded RNA, approximately 12.300 nucleotides in length. The CSFV genome encodes a polyprotein composed of 3.898 amino acid residues, which is cleaved by cellular and viral proteases to produce 12 major protein products, including N^{pro}, C, E^{rns}, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B *(Fig. 2)*. These proteins play crucial roles in the virus's replication, assembly, and evasion of the host immune response.

The E2 and E^{rns} proteins of CSFV play a key role in the virus's immune evasion. The E2 protein is the main immunogen, capable of inducing the host to produce neutralizing antibodies, while the E^{rns} protein helps the virus escape by suppressing the host's antiviral immune response. Additionally, the p7 protein of CSFV is believed to play an important role in the virus's assembly and release process, with functions in regulating the intracellular environment and promoting the formation of the viral envelope.

The transmission routes of CSFV mainly occur through direct contact with infected pigs, contaminated environments, and biological vectors. After infection, CSFV can trigger an immune response in pigs, leading to various clinical symptoms, including high fever, reduced appetite, and bleeding tendencies, which can result in significant mortality in severe cases. This virus has caused serious economic losses globally, especially in the pig farming industry, making research and vaccine development for CSFV of significant practical importance. In recent years, researchers have focused on the variants of CSFV and their prevalence in different regions to better understand the epidemiological characteristics of the virus and to formulate effective prevention and control measures.

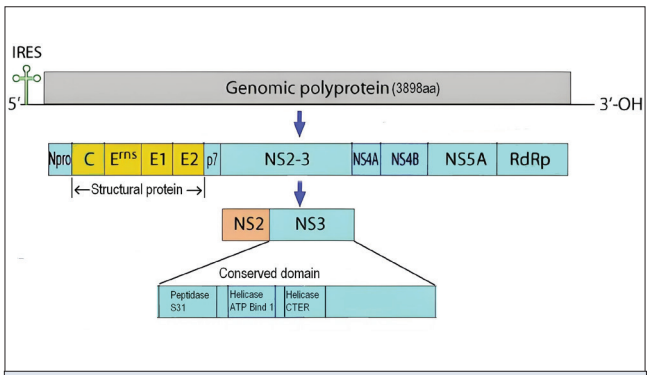

Fig 2. The genome map of CSFV. The genomic structure comprises three parts: the 5'-untranslated region (UTR) containing the internal ribosome entry site (IRES), the polyprotein open reading frame (ORF), and the 3'-UTR. In the large polyprotein ORF, Non-structural proteins are labeled in blue, and structural proteins are color-coded in yellow

Structure and Function of NS3

The NS3 protein exists mainly in two forms after classical swine fever virus (CSFV) infects host cells: as

an NS2-3 protein complex and as a monomeric NS3. The NS2-3 complex is a key molecule in the viral life cycle, particularly in the replication and assembly of viral particles [5]. According to research, the molecular weight of the NS3 protein is approximately 80 kDa, and its formation results from the cleavage of the NS2-3 protein complex by NS2. The NS3 protein plays an important role in viral replication and is also involved in the processing of precursor proteins, providing necessary conditions for viral maturation and assembly.

The NS3 protein of CSFV is a multifunctional enzyme that possesses both RNAse and protease activities. NS3 is primarily responsible for viral replication and the processing of precursor proteins, which are essential for viral maturation and assembly. Structurally, NS3 consists of two main domains: NS3a and NS3b, where NS3b is mainly associated with the replication of viral RNA, while NS3a plays a role in viral cell infection and interaction with the host immune response. The functions of NS3 are closely related to its structure, making the study of its amino acid sequence, domains, and tertiary structure crucial. The high conservation of the NS3 amino acid sequence indicates its importance in the viral life cycle. A deeper understanding of the structure and function of NS3 will aid in the development of antiviral drugs targeting flaviviruses.

The multifunctional characteristics of NS3 make it a central subject in the study of CSFV biology, and a thorough understanding of its structure and function is significant for developing antiviral strategies.

Amino Acid Sequence and Conserved Nature of NS3

The amino acid sequence of the NS3 protein exhibits a high degree of conservation among different viruses in the Flavivirus family, which is an important basis for its function. Studies have shown that the amino acid sequence of NS3 contains multiple functional regions that play key roles in the virus's life cycle. Conserved amino acid residues are not only crucial for the structural stability of NS3 but are also closely related to its enzymatic activity. For example, the serine protease activity of the NS3 protein depends on specific amino acid sequences, and the conservation of these sequences provides important evidence for the development of inhibitors targeting NS3. Furthermore, the conserved amino acid sequences may also influence the subcellular localization of NS3 and its interactions with host factors, thereby affecting viral replication and pathogenicity.

These conserved features make NS3 a potential target for vaccines and antiviral drugs, and studying the variations in its amino acid sequence and their impact on function is significant for understanding the virus's adaptability and evolution *(Fig. 3)*.

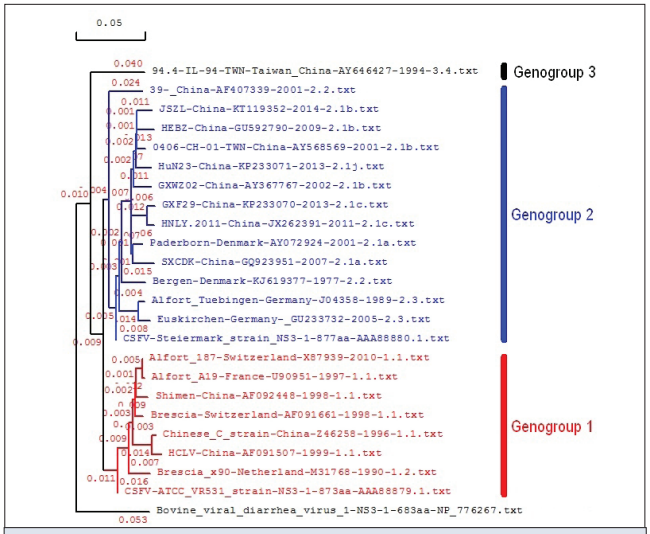

Fig 3. The phylogenetic tree created with the Maximum Likelihood method based on CSFV NS3 protein sequences from different clinical isolates. The phylogenetic tree was constructed by the Maximum Likelihood method with DNAMAN version 8 software after full sequences alignments, and the branch length indicated at the branch nodes were evaluated using 1.000 bootstrap replications. For CSFV, GenBank accessing numbers are listed after the name of each virus isolate's name and location. Besides, the Bovine Viral Diarrhea Virus (BVDV) NS3 protein sequence was used as an outgroup

The Conserved Domains of NS3 and Their Biological Functions

The functions of the NS3 protein can be divided into several domains, including the serine protease domain, the nucleoside triphosphatase domain, and the RNA-activated helicase domain. Each domain carries out specific biological functions, allowing NS3 to play multiple roles in the viral life cycle. The serine protease domain is responsible for the cleavage of viral polyproteins, the nucleoside triphosphatase domain is involved in RNA synthesis and energy metabolism, while the RNA helicase domain plays a crucial role in the replication and transcription of viral RNA [6]. These domains of the NS3 protein not only participate in viral replication and assembly but may also promote the survival and spread of the virus by regulating the immune response of host cells. The interactions and coordinated functions of these domains are essential for the efficient replication of the virus. Therefore, in-depth research on the domains of NS3 and their functions is of great significance for understanding the pathogenic mechanisms of CSFV and developing targeted therapeutic strategies.

NS3 Serine Protease Functional Region

The serine protease functional region of NS3 is one of its most critical domains, responsible for cleaving viral precursor polypeptides to generate mature viral proteins. The catalytic mechanism of this functional region involves several conserved amino acid residues, including serine, histidine, and aspartic acid, which play a central role in the

catalytic process. Studies have shown that the activity of the NS3 serine protease not only depends on the presence of these amino acids but is also influenced by their spatial conformation. Furthermore, the interaction between NS3 and its cofactor NS2B is crucial for the activity of the serine protease, as NS2B can enhance the enzymatic activity of NS3, thereby increasing the efficiency of viral replication. Additionally, the serine protease function of NS3 may also play a role in regulating the host immune response, which in turn affects the pathogenicity and transmissibility of the virus [7].

The Nucleoside Triphosphatase (NTPase) Functional Domain of NS3 Protein

The NTPase functional domain of NS3 is responsible for hydrolyzing nucleoside triphosphates, providing energy for RNA synthesis and viral replication. The activity of this functional domain is closely related to the structure of NS3, particularly its ability to bind ATP. Studies have found that the NTPase activity of NS3 is influenced by conformational changes in its domains, which affect the binding and hydrolysis efficiency of ATP [8]. Additionally, the activity of NTPase may also be related to the intracellular localization of NS3 and its interactions with other viral proteins, thereby impacting the overall viral replication capacity.

The NTPase functional domain of the NS3 protein plays a crucial role in the viral replication process. Research by Wen et al.[9] revealed the NTPase activity of NS3 under specific polynucleotide stimulation and its reaction conditions, providing important clues for a deeper understanding of the replication mechanism of CSFV. The activity of NTPase not only affects the RNA synthesis of the virus but is also related to the metabolic activities of host cells. Therefore, studying the regulatory mechanisms of NTPase can help identify new antiviral targets, providing a theoretical basis for the development of effective antiviral drugs.

RNA Helicase Functional Region of NS3 Protein

The RNA helicase functional region of NS3 is also crucial in the viral RNA replication process. The NS3 protein possesses RNase activity, enabling it to unwind RNA strands during viral replication, facilitating RNA replication and transcription. Specifically, NS3 can unwind viral RNA through its helicase activity, maintaining a single-stranded state during replication, thereby promoting RNA synthesis and translation. This process is vital for the viral life cycle, as the unwinding of viral RNA is a prerequisite for replicating and expressing the viral genome.

Research shows that the helicase activity of NS3 is influenced by the spatial configuration of its domains, and specific amino acid residues are critical for the catalytic efficiency of the helicase [10]. Additionally, the helicase activity may be regulated by the host cell environment following viral infection, and NS3 can interact with other viral proteins and host cell factors to modulate the viral replication environment. For example, the NS3 of Zika virus supports the assembly of viral replication factories by utilizing the host's antiviral RNase L protein, thereby enhancing the virus's replication capacity [11]. This complex interaction network allows NS3 to play multiple roles in viral replication.

By analyzing the interaction between NS3 and NS5B, Wen et al.[12] discovered how the helicase and NTPase activities of NS3 are regulated differently, thereby enhancing the activity of CSFV's RNA-dependent RNA polymerase in viral replication. The studies by Wen et al.[12] and Sheng et al.[13] provided potential targets for future antiviral strategy development, indicating that interventions targeting the helicase activity of NS3 may effectively inhibit viral replication.

Studies of the Tertiary Structure of NS3

The three-dimensional structure analysis of the NS3 protein reveals its complex spatial conformation and functional domains, including the protease active site and helicase active site. NS3 typically forms a functionally complete protease complex with its cofactor NS2B, which plays a key role in the virus's life cycle.

Using high-resolution X-ray crystallography and nuclear magnetic resonance (NMR) techniques, scientists have conducted detailed structural analyses of the NS3 protein from CSFV. These studies reveal the spatial conformation of NS3 and how it binds to substrates, providing a structural basis for understanding NS3's function [14].

Additionally, researchers have successfully resolved the NS3 structures of various viruses. For example, the crystal structure of the NS3-like helicase from the Alongshan virus provides an in-depth understanding of the protein's function [15]. The tertiary structure of NS3 not only showcases the interactions between its functional regions but also reveals how its activity can be regulated by altering specific amino acid residues. These studies provide an important structural basis for the design and development of specific inhibitors targeting NS3, with significant clinical application prospects [16]. Research indicates that effective inhibitors can be developed to combat viral infections by targeting specific domains of NS3 [17].

Furthermore, studies have found that the conformation of NS3 may change during transmission, which could affect the efficiency of its enzymatic activity. These three-dimensional structural studies lay the groundwork for

further exploration of NS3's role in the virus life cycle and provide important information for the development of antiviral drugs targeting NS3 [10,15].

Factors Affecting the Subcellular Localization of NS3 Protein

The subcellular localization of the NS3 protein is crucial for its function, and the factors influencing its localization include the host cell environment, the viral infection status, and the structural characteristics of NS3 itself. Studies have shown that certain regions of NS3 can interact with the internal membrane structures of host cells, thereby affecting its distribution and localization within the cell [18]. Research indicates that different localizations of NS3 within the cell may influence its enzymatic activity and interactions with other viral proteins [19]. By regulating the subcellular localization of NS3, the virus can optimize its replication and assembly processes, thereby enhancing infection efficiency. Therefore, exploring the factors that affect NS3 subcellular localization can help in understanding the pathogenic mechanisms of CSFV and provide insights for developing new antiviral strategies [20].

Other Studies on NS3 Protein Function

In addition to the aforementioned functions, the NS3 protein is also involved in multiple biological processes, including regulating the immune response of host cells and affecting cell signaling pathways. Studies have found that NS3 can inhibit the host's antiviral response by interacting with host cell proteins, thereby promoting the survival and spread of the virus [18]. Furthermore, the function of NS3 is also related to the adaptive evolution of the virus, with research indicating that mutations in NS3 may affect the virulence and transmissibility of the virus [21]. Further studies suggest that NS3 may perform different functions in different cell types, providing new insights into the complex biological characteristics of CSFV. Through in-depth research on NS3 function, it is expected that more effective antiviral treatment strategies can be developed in the future [22].

The Role of NS3 in CSFV Replication

The NS3 protein plays a crucial role in the viral replication process, especially in the replication mechanisms of flaviviruses (e.g. hepatitis C virus, HCV). Research indicates that NS3 is not only one of the main enzymes of the virus but also participates in the synthesis and processing of viral RNA.

The Relationship Between NS3 and Viral RNA Synthesis

The non-structural protein NS3 of classical swine fever virus (CSFV) plays a vital role in viral replication. Research by Sheng et al.[13] shows that NS3 is closely related to viral RNA synthesis, particularly its interaction with the viral 3' non-coding region, which is considered crucial for the replication of the CSFV genome. Studies indicate that NS3 can promote RNA synthesis by interacting with NS5, which acts as an RNA polymerase and can efficiently synthesize viral RNA with the assistance of NS3. This interaction is essential for viral replication, as RNA synthesis is one of the core steps in the viral life cycle [23]. Additionally, the helicase activity of NS3 helps to unwind RNA secondary structures, providing a template for the RNA polymerase, further enhancing its importance in viral replication. Therefore, exploring the relationship between NS3 and viral RNA synthesis contributes to understanding the replication mechanism of CSFV and provides a theoretical basis for developing antiviral strategies against CSFV [13].

The Mechanism of NS3 in Viral Genome Replication

The role of NS3 in viral genome replication is relatively complex, involving multiple steps and interactions. First, NS3 interacts with NS5 (RNA polymerase) to form an effective replication complex, thereby enhancing the efficiency of RNA synthesis [23]. Studies have shown that structural changes in NS3 play a key role in RNA binding and catalytic reactions, effectively regulating the replication and transcription of viral RNA. In addition, NS3 alters the lipid environment within host cells by cleaving relevant host cell enzymes, providing the necessary conditions for viral RNA replication. During this process, NS3 forms a complex with other non-structural proteins (such as NS2B) to participate in RNA synthesis and processing, ensuring the integrity and replication efficiency of the viral genome. In summary, NS3 is not only an important enzyme in viral genome replication but also forms a complex regulatory network through interactions with other proteins to ensure effective viral replication and transmission.

The Role of NS3 in the Virus Assembly Process

NS3 plays an important role in the assembly process of CSFV. Research has found that NS3 participates in the formation and release of viral particles through interactions with other non-structural proteins. Specifically, the surface structure of NS3 can interact with viral membrane proteins, promoting the maturation and assembly of viral particles. This interaction not only affects the morphology of the virus but may also influence its infectivity. Therefore, the function of NS3 is indispensable in the virus's life cycle. Additionally, the endogenous self-cleavage characteristics of NS3 allow for functional separation. A study by Lamp et al.[7] revealed that the autocatalytic cleavage process in NS3 produces fragments with enzymatic activity, which are crucial for viral replication and assembly. This process

not only affects the structural integrity of the virus but may also regulate the efficiency of viral assembly. The biological significance of the self-cleavage event provides a new perspective on the multifunctional polymerase of the Flaviviridae family. These findings emphasize the multiple functions of NS3 in the viral life cycle, particularly its importance in the processes of viral assembly and maturation, making it an important target for studying viral assembly mechanisms and providing potential targets for future vaccine and antiviral drug development.

Interaction Between NS3 and NS5 During Viral Replication

There is a significant interaction between NS3 and NS5B during the replication of CSFV. A study by Xiao et al.[24] found that the full-length form of NS3 enhances IRES-mediated translation more effectively than the truncated form, demonstrating the key role of NS3 in viral proliferation. Furthermore, NS5B can significantly promote the stimulatory effect of NS3 on translation, providing new insights into the understanding of viral proliferation mechanisms. Sheng et al.[25] further explored the importance of NS3, NS5A, and NS5B in CSFV replication through mutation and complementation analysis, revealing the critical interaction between NS3 and NS5B. Wang et al.[26] indicated that NS3 binds to NS5B through its protease domain, enhancing the activity of the RNA-dependent RNA polymerase, highlighting the important function of NS3 in the CSFV replication life cycle. Wang et al.[27] identified two NS3 binding sites by deleting the terminal sequences of NS5B and explored how this interaction enhances the RNA-dependent RNA polymerase activity of NS5B. These studies provide direction for the development of antiviral drugs, revealing the complex interactions between NS3 and NS5 and their importance in viral replication.

The Function of NS3 in Viral Protein Translation

NS3 also plays an important role in viral translation. Research by Deng et al.[20] showed that NS3 can affect the translation efficiency of viral RNA through interactions with translation-related factors, thereby influencing the expression levels of viral proteins. This regulatory mechanism is crucial for the survival and reproduction of the virus. Additionally, a study by Zhu et al.[2] indicated that NS3 is an IRES-binding protein that enhances IRES-mediated translation by binding to the CSFV IRES. This mechanism suggests the importance of NS3 in viral replication, indicating that it not only participates in RNA synthesis but also directly affects the translation process of viral proteins. The IRES-binding characteristics of NS3 may provide the virus with an advantage for efficient translation within host cells, thereby promoting rapid viral proliferation. This finding offers new insights into the replication mechanism of CSFV and provides potential targets for future therapeutic strategies.

The NS3 Protein is Associated with Cytopathic Effects of CSFV *In vitro*

The NS3 protein plays a key role in the cytopathological characteristics of CSFV. A study by Aoki et al.[28] revealed that the accumulation of NS3 is closely related to the occurrence of cytopathic effects (CPE) in cell cultures, especially during infections in serum-free media. The degree of cytopathic effects is positively correlated with the expression level of NS3, indicating that NS3 may directly or indirectly act on important host cell proteins, leading to cell damage. Research by Xu et al.[29] further confirmed that the expression of NS3 is closely related to significant changes in cell morphology and increased apoptosis rates, providing important clues for understanding the pathogenic mechanism of CSFV and the formation of persistent infections. The study by Xu et al.[29] revealed the core role of NS3 in inducing apoptosis and cytopathic effects by transfecting the classical swine fever virus NS3 gene. The results showed that the expression of the NS3 protein is closely related to significant changes in cell morphology and increased apoptosis rates, affecting the cytopathic effects in PK-15 cells. This helps to understand the pathogenic mechanism of the swine fever virus and the formation mechanism of persistent infections. NS3 and NS2 can be detected in cells infected with cytopathic strains of the virus, while non-cytopathic strains only express the NS2-NS3 polyprotein in their infected host cells. Therefore, the non-structural protein NS3 can serve as a specific marker protein for cytopathic classical swine fever virus at the protein level. NS3 may directly or indirectly act on important host cell proteins, leading to cell damage. In cells infected with cytopathic CSFV, the more pronounced the cytopathic effects, the higher the detected NS3 content, indicating a close relationship between NS3 and the occurrence of CPE. As a specific marker protein for cytopathic classical swine fever virus at the protein level, the role of NS3 in the occurrence of CPE provides a new research direction for vaccine development.

Interaction of NS3 with Host Immune Response

Impact of NS3 on Host Immune Evasion Mechanisms

The NS3 protein plays a key role in *Flaviviridae* infections, interfering with the host's immune response through various pathways, allowing the virus to persist and spread within the host. For example, the HCV NS3/4A complex can cleave critical immune regulatory factors, disrupting cytokine signaling pathways. Relevant studies indicate that

NS3/4A can reduce the expression of pro-inflammatory cytokines by inhibiting the activation of NF-κB, thereby weakening the host's inflammatory response and antiviral immune response [30]. In the case of Zika virus (ZIKV), NS3 can also enhance the virus's immune evasion capability by interacting with intracellular adaptor proteins MAVS and MITA to inhibit the production of interferons [31]. Additionally, HCV NS3 has been found to promote T cell exhaustion, leading to a weakened immune response in chronic HCV infection patients, which in turn affects the ability to clear the virus [32]. Cao et al.[22] constructed a lentiviral vector expressing CSFV non-structural proteins and infected porcine monocyte-derived macrophages (pMDMs), finding that NS3 significantly downregulated the expression of Toll-like receptors (TLRs). This finding suggests that NS3 may help the virus escape host immune surveillance by inhibiting the activation of TLR signaling pathways, thereby interfering with the initiation of the innate immune response. Understanding these immune evasion mechanisms is crucial for developing new vaccines and therapeutic strategies, as it reveals how viruses exploit the host's immune system to promote their own replication and spread.

Researches on NS3-Induced Immune Response

The NS3 protein plays an important role in the immune response of pigs to classical swine fever. Research by Rau et al.[33] indicated that recombinant NS3 protein can effectively induce the production of CD8+ cytotoxic T cells, suggesting that NS3 may serve as an effective immunogen. A study by Vazquez et al.[34] found that NS3 enhances the activation of specific T cells by promoting antigen presentation and cytokine production through the activation of dendritic cells (DCs) and macrophages. Using recombinant vaccines and natural adjuvants, Hajikhezri et al.[35] observed that HCV NS3 antigen could effectively activate T cell responses, inducing a strong Th1-type cytokine response. Furthermore, Pouriayevali et al.[36] found that the immunogenicity of HCV NS3 is influenced by its conformation and modification state; optimizing the expression and purification conditions of HCV NS3 can further enhance the strength and durability of the induced immune response. These studies provide experimental evidence for further exploring the potential of NS3 as a vaccine component, particularly in how to enhance the immunogenicity of NS3 during vaccine development, which will be an important research direction.

Research by Voigt et al.[37] found that although vaccination with NS3 protein can stimulate the immune response in piglets, it does not provide effective protection against lethal CSFV challenge infection, indicating that further optimization of the delivery system is needed in vaccine design to enhance its protective effect. Nevertheless, in both *in vivo* and *in vitro* experiments, the combination of NS3 with E2 protein showed good immunogenic effects, potentially providing a breakthrough for the development of new vaccines [33]. These studies provide important experimental foundations for understanding the role of NS3 in immune responses and for vaccine development.

Interaction of NS3 with Host Proteins

The Interaction Between NS3 Protein and Host Cell Proteins Promotes Viral Replication and Immune Evasion

Research by Lv et al.[38] found that the interaction of CSFV NS3 protein with TRAF5 not only promotes viral replication but also affects the host's immune response by activating the p38 MAPK pathway. Additionally, TRAF6 has been identified as an inhibitor of CSFV replication; Lv et al.[39] found that TRAF6 inhibits CSFV replication by enhancing the activity of the NF-κB signaling pathway. These studies reveal the complex interplay between NS3 and host cell signaling pathways, providing new insights into the immune evasion mechanisms of CSFV.

NS3 Achieves Evasion Not Only by Suppressing Immune Responses but Also by Interacting with Host Cell Factors to Influence the Immune Environment

For example, the NS3 of Zika virus can inhibit the activation of the NLRP3 inflammasome, thereby reducing the release of pro-inflammatory cytokines, a mechanism that allows the virus to evade immune surveillance within the host [40]. Furthermore, NS3 may also affect the host's immune response by regulating the expression of cytokines. For instance, research by Latanova et al.[41] showed that HCV NS3 can increase the secretion of interleukin-1β, which may be related to its regulation of the host immune environment. This regulatory effect not only affects the host's immune response but may also create favorable conditions for the virus's persistent infection. Therefore, the role of NS3 in host immune evasion is multifaceted, highlighting its importance as a potential therapeutic target.

The NS3 Protein Regulates the Host's Signaling Pathways Through Interactions with Various Intracellular Proteins

For example, research by Cao et al.[42] demonstrated that Japanese Encephalitis Virus NS3 can interact with intracellular heat shock proteins (such as Hsp40), affecting viral replication and the host cell's stress response. Additionally, research by Abdullah et al.[30] found that HCV NS3 can interact with various kinases, regulating their activity and thereby influencing cell proliferation and apoptosis. These interactions not only support the survival of the virus but also interfere with the host's

immune response, forming a complex network of virus-host interactions.

The Interaction Between NS3 and Host Cell Proteins Also Aids in the Spread of the Virus Within the Host

The interaction of NS3 with host cell proteins not only affects the immune response but also plays a crucial role in viral transmission. Research by Silva et al.[18] indicates that Dengue virus NS3 can inhibit the enzymatic activity of host cell glycolytic enzymes (such as GAPDH) through interaction, thereby affecting cellular energy metabolism and promoting viral replication and spread. Additionally, the study by Palacios-Rápalo et al.[43] shows that the nuclear localization of Dengue virus NS3 may also influence the assembly and release of viral particles in human host cells (Huh7 cells), further enhancing its transmission capability within the host. Therefore, in-depth research on the interaction mechanism between NS3 and host proteins is of great significance for understanding the biological characteristics of the virus and developing new antiviral strategies.

The Binding of NS3 Protein to Host Cell Proteins May Negatively Regulate Viral Proliferation and Promote the Degradation of Viral Proteins

For example, Deng et al.[20] found that the host cell protein PSMB10 can interact with NS3 protein, inhibiting the proliferation of CSFV and mediating the degradation of NS3 through the ubiquitin-proteasome pathway. This finding not only reveals the complex interactions between the virus and the host but also provides new targets for the prevention and control of CSF. These studies emphasize the importance of NS3 in the viral transmission process, and further research will help uncover its specific mechanisms in host immune evasion and viral spread.

Research of NS3 as A Vaccine Target

CSFV has Three Genes That Can Induce the Production of CSF Antibodies, Namely E0, E2, and NS3

Among them, E0 and E2 proteins induce the body to produce protective antibodies, while NS2-3 antibodies do not have virus neutralization activity. CSF is an important viral disease that has severely impacted the pig farming industry. Studies have shown that the E0 and E2 proteins of CSFV can effectively induce the body to produce protective antibodies, thereby providing immune protection. However, as a non-structural protein, the antibodies produced by NS3 have a weaker ability to neutralize the virus, limiting its application in vaccine development. Nevertheless, NS3 remains an important research subject because it plays a key role in the virus's replication and infection processes. An effective vaccine needs to combine multiple antigens to maximize the immune system's response, so in-depth research on NS3 will help understand its potential role in vaccine design, providing new ideas and strategies for future vaccine development.

NS3-Based Vaccine Candidate Strategies

The NS3 protein is an important non-structural protein of various viruses, such as the hepatitis C virus and dengue virus, and has become a popular target for vaccine development due to its critical role in viral replication and pathophysiological processes. Studies have shown that NS3-based vaccine strategies can effectively induce specific immune responses. For example, using a necrotic dendritic cell vaccine expressing HCV NS3 can significantly enhance T cell responses, demonstrating good immunogenicity and protective effects. Additionally, DNA vaccines expressing HCV NS3 protein have also shown enhanced cellular immune responses in mouse models, providing strong support for NS3 as a vaccine target. Combining different immune adjuvants, such as the N-terminal heat shock protein gp96, can further improve the immune response of HCV NS3 vaccines, inducing strong Th1-type cytokine production [35]. These studies indicate that NS3-based vaccine candidate strategies have great potential in eliciting cellular immunity and antiviral protection.

Due to the importance of NS3, researchers have targeted it for vaccine development. In the development of existing QS vaccines and genetically engineered vaccines, NS3 is considered a potential protective antigen. Scientists hope to enhance the effectiveness of vaccines by improving the host's immune response to NS3. There have been several successful cases in the research of NS3 as a vaccine target. For instance, using modified vaccine vectors to express NS3 antigens has shown enhanced immune responses and better protective effects. Furthermore, researchers have explored combining different adjuvants and immune enhancers to improve the specific immune response to NS3. Through these strategies, researchers aim to develop more effective vaccines to combat CSFV and other related viruses [36].

Potential Applications of NS3 in Vaccine Design

The NS3 protein plays a crucial role in the immunogenicity of vaccines. Firstly, the Zika virus ZIKV NS3 can act as a potent T cell antigen, inducing the activation and proliferation of CD8+ T cells, which is essential for clearing viral infections. Secondly, HCV NS3 also exhibits good immunogenicity and antigen presentation capability, able to combine with various immune adjuvants to enhance the strength and durability of the immune response [36]. Thirdly, the use of a combined

vaccine strategy with modified NS1 and NS3 has shown significant immune enhancement effects, providing new ideas for the development of vaccines against dengue virus. In addition, the structural features and functional characteristics of NS3 make it an ideal candidate for designing multivalent vaccines that can target multiple viral strains simultaneously. In summary, NS3 is not only an important component of viral replication but also a significant target in vaccine development, and its role in immunogenicity ensures the effectiveness of vaccines.

Progress in NS3 Basic Vaccine Research

What is the potential of the NS3 protein in expression and surface display in insect host cells? Xu et al.[44] successfully displayed the CSFV NS3 protein on the viral envelope using a modified BACBd virus system, significantly enhancing antibody production in mouse models, providing a solid foundation for novel vaccine strategies. This study indicates that displaying CSFV NS3 through modified viral vectors can effectively stimulate the host's immune response. Additionally, researchers are exploring other expression systems and strategies to further enhance the immunogenicity and protective effects of NS3. For example, using natural adjuvants and cell-penetrating peptides as carriers simultaneously can improve the stability and biocompatibility of NS3, thereby enhancing the overall efficacy of the vaccine [45]. These studies provide new directions for the application of NS3 as a vaccine target and lay the groundwork for future vaccine development.

Future Research Directions for NS3

Development of Novel Inhibitors

NS3 protease is a key enzyme in the replication process of various viruses, such as the hepatitis C virus and dengue virus. Therefore, developing novel inhibitors targeting NS3 has significant clinical implications. In recent years, researchers have identified a series of promising new small molecule inhibitors through virtual screening and structural biology methods. For example, studies have shown that consensus scoring-based virtual screening can effectively discover inhibitors targeting the NS3 protein, which exhibit good inhibitory activity *in vitro* [46]. Additionally, the development of inhibitors against HCV NS3 is not limited to small molecules; biopharmaceuticals such as monoclonal antibodies have also shown potential applications in research [47]. Future research could focus on optimizing the pharmacokinetic properties of these inhibitors to enhance their efficacy and safety in clinical applications. Furthermore, combining computational drug design with high-throughput screening technology may accelerate the discovery and development of novel NS3 inhibitors.

Role of NS3 in Viral Mutation

The NS3 protein plays multiple roles in the viral life cycle, particularly in viral mutation and adaptation. Research indicates that NS3 is involved not only in viral protein processing and replication but may also influence the virus's ability to withstand adversity, such as the development of drug resistance [48]. In the case of the hepatitis C virus, mutations in NS3 can lead to resistance to protease inhibitors, posing challenges for antiviral therapy [49]. Moreover, mutations in NS3 may also affect its interactions with host cell factors, thereby altering the virus's pathogenicity and transmission capabilities [50]. Future research should focus on the mechanisms of NS3 mutations and their impact on viral adaptability, which will provide an important theoretical basis for developing new therapeutic strategies. Additionally, monitoring NS3 mutations can guide vaccine design and public health interventions to address the challenges posed by viral mutations.

There are Still Many Unresolved Mysteries Regarding CSFV NS3

Future research needs to further explore the specific mechanisms of NS3 in the interaction between the virus and the host, as well as the differences in immune responses among different pig breeds. Furthermore, developing novel vaccines based on NS3 and assessing their safety and efficacy in practical applications will be key focuses of future research.

Conclusion

Generally speaking, the non-structural protein 3 (NS3) of the CSFV plays a central role in viral biology, host immune response, and vaccine development *(Table 1)*. With a gradual deepening understanding of NS3's functions, we have made significant progress in the field of CSFV research, particularly in elucidating its role in viral replication, host immune evasion mechanisms, and vaccine design. However, despite numerous findings, there remain many unresolved mysteries related to NS3, presenting new challenges for future research.

Future research directions should focus on revealing the specific mechanisms of NS3 in the interactions between the virus and the host, as well as exploring the differences in immune responses among different pig breeds. This not only helps us understand how the virus affects the host's immune response but also provides a foundation for developing more targeted vaccines. Additionally, the development of novel vaccines based on NS3 will be an important approach to addressing CSFV outbreaks. Researchers need to strengthen the design of preclinical and clinical trials to ensure the safety and efficacy of new vaccines, thereby ensuring the sustainable development of the pig industry.

Table 1. *Summary of Classical Swine Fever Virus (CSFV) NS3 protein's functions*

Category of NS3 Function	Functional Description	References
Regulation of viral translation	NS3 regulates viral RNA translation, affecting viral protein expression levels	[2]
Protease activity	NS3 exhibits serine protease activity, involved in cleaving viral polyproteins, crucial for viral replication	[5]
Virus assembly	NS3 is involved in viral particle assembly, affecting virus morphology and infectivity	[7]
NTP enzyme activity	NS3 has NTPase activity, providing energy for RNA synthesis	[9]
Three-dimensional(3D) structural characteristics of the protein	NS3 contains catalytic triad (His-1663, Asp-1686, Ser-1694), and its resolved 3D structure aids drug design	[14,15]
RNA helicase activity	NS3 possesses RNA helicase activity, able to unwind secondary structures of viral RNA, facilitating RNA replication and transcription	[23]
Association of NS3 protein with the pathogenicity of CSFV	NS3 mutations may alter viral replication efficiency in host cells, correlating with virulence	[25,50]
Formation of replication complexes	NS3 interacts with proteins like NS5B to form replication complexes, enhancing RNA-dependent RNA polymerase activity.	[26]
Regulation of apoptosis	NS3 accumulation in infected cells may affect viral pathogenicity by modulating apoptosis	[29]
Immunogenicity differences	NS3 induces host antibody production, but its epitopes are less conserved than E2, affecting detection specificity	[32,36]
Immune evasion	NS3 suppresses host immune responses through various mechanisms, aiding viral immune evasion	[45]

When balancing different research perspectives and findings, we should fully consider factors such as experimental design, sample selection, and data analysis. In some studies, there may be differences in the interpretation of NS3 functions, especially under different experimental conditions or pig breed backgrounds. Therefore, focusing on multi-center, large-scale collaborative research will help integrate various research results and form more comprehensive and consistent conclusions.

In conclusion, research on NS3 not only provides us with an opportunity to gain a deeper understanding of CSFV biology but also offers new ideas for vaccine development and improvements in control strategies for the pig industry. We look forward to achieving greater breakthroughs in these areas to address the ever-changing viral challenges.

Highlight Keypoints

- CSFV NS3 has three conserved domains in biology, including serine protease, nucleoside triphosphatase (NTPase), and RNA helicase functional regions.
- NS3 plays a key role in the replication, pathogenicity, and immune evasion of CSFV.
- NS3 interacts with the host cells immune response.
- NS3 can serve as a vaccine target and has potential applications in vaccine design.

Declarations

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