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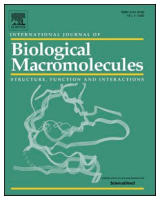

Antigenicity, epitope mapping, and intracellular distribution of the NSP7α protein of porcine reproductive and respiratory syndrome virus

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is an important pathogen that causes huge economic losses to the global pig industry. Nonstructural protein 7α (NSP7α) of PRRSV is highly conserved among different lineages of PRRSV and could be a potential target for the development of detection methods. In this study, NSP7α was expressed in prokaryote (*Escherichia coli*) and purified. An NSP7α-ab-ELISA detection method was established, the NSP7α-ab-ELISA has 93.1 % coincidence rate with IDEXX PRRS X3 ab test kit. NSP7α antibody was detected in pig serum by ELISA 14 days following PRRSV infection. Three monoclonal antibodies (4H9, 3F2, and C10) against NSP7α prepared by a hybridoma technique were used for epitope mapping by indirect immunofluorescence. The 4H9, 3F2, and C10 antibodies all recognized the C-terminal 72–149 amino acid region of NSP7α. 4H9 reacted with amino acids 135–143, but 3F2 and C10 did not react with any truncated polypeptide. In addition, by using the monoclonal antibodies, NSP7α was localized solely in the cytoplasm, while the N protein was distributed in the cytoplasm and nucleus. The collective findings of the antigenicity and epitope of NSP7α will be helpful for understanding the antigenicity of NSP7α and developing PRRSV diagnostic methods.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most common diseases affecting pigs. PRRS is characterized by abortion, stillbirth, and mummified fetuses in pregnant sows, as well as a respiratory syndrome in piglets [1]. PRRS virus (PRRSV) is a positive-sense single-stranded RNA virus that belongs to the *Nidovirales* order, *Arteriviridae* family, and *Betaarterivirus* genus. There are two genotypes, PRRSV-1 and PRRSV-2. Based on phylogenetic analysis of the open reading frame (ORF)5 sequence, PRRSV-2 has been divided into nine lineages (L1-L9) [2]. The PRRSV genome is approximately 15.4 kb in length. The genome contains at least nine ORFs. ORF1a encodes the polypeptide pp1a, which is cleaved into eight nonstructural proteins (NSP1-NSP8) by viral proteases. Through a programmed ribosomal frameshift of −1, ORF1a and ORF1b encode pp1b, which is further hydrolyzed to NSP1-NSP12. NSPs are essential for viral replication. ORF2-ORF7 encode the viral structural proteins, GP2-GP5, M, and N, respectively. ORF2b encodes the E protein.

NSP7 is essential for PRRSV replication. Mutation and deletion analyses demonstrated that NSP7 was critical for PRRSV rescue [3]. NSP7 is involved in the formation of replication transcription complex (RTC), by interacting with NSP5, NSP9, and NSP12 of PRRSV [4,5]. NSP7 is cleaved at the 149E/150 N position by NSP4 to form NSP7α and NSP7β [6]. In addition to its involvement in viral replication, NSP7 can interact with host factors and regulate host innate immune responses. The mRNA levels of interferon regulatory factor 7 (IRF7) mRNA level is downregulated in NSP7-overexpressing cells [7]. NSP7 also downregulates a helicase-like transcription factor, which further promotes the transcription of the E3 ubiquitin ligase RING finger protein 122 (RNF122) to inhibit the innate immune response [8]. The structure of NSP7α resolved by nuclear magnetic resonance contains three α-helices and six β-sheet ordered as α1-β1-α2-α3-β2-β3-β4-β5-β6 [9]. However, the biological function of NSP7α remains unclear. The monoclonal antibody against NSP7 is an important tool and useful for the investigation of the function and mechanism of NSP7 in PRRSV replication.

In this study, the antigenicity and intracellular distribution of NSP7α were probed, and epitope mapping was performed using anti-NSP7α monoclonal antibodies. NSP7α antibody was detected in clinical pig

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serum and comparing with commercial detection kit. The results deepen the understanding of the antigenic structure of NSP7α.

2. Materials and methods

2.1. Cells, virus, serum, and experimental animals

Marc-145, BHK-21, and SP2/0 cells were cultured in DMEM (Sigma-Aldrich, USA) with 10 % fetal bovine serum (FBS) at 37 °C in an atmosphere of 5 % CO_2. The PRRSV-2 strains, including the lineage 8 (L8) PRRSV HuN4 strain (GenBank no. EF635006), S043 strain of the L1 lineage (GenBank No. OM201173), the GD59 strain of L3 lineage (GenBank No. OM201184), the modified live vaccine strain lngelvac MLV of L5 lineage, and the PRRSV-1 DV strain were maintained in our laboratory. Sera were collected from piglets infected with strain S043 at 0, 3, 7, 10, and 14 days post-infection (dpi) and maintained in our laboratory [10]. Six-week-old male BALB/c mice were purchased from the Laboratory Animal Center of Harbin Veterinary Research Institute.

2.2. Plasmid construction, expression, and purification of NSP7α protein

The NSP7α of PRRSV was amplified by reverse transcription-polymerase chain reaction (RT-PCR) with forward primer (5′-AGAAGGAGATATA*CATATG*TCGCTGACTGGTGCCCTCG-3′) and reverse primer (5′-TCAGTGGTGGTGGTGGTGGTGCTCCAGAACTTTCGGTGGGA-3′) based on PRRSV HuN4 strain. NSP7α was cloned into pET24a(−) via *Nde* I and *Xho* I, resulting in a recombinant prokaryotic expression plasmid pET24-His-NSP7α. In addition, NSP7α was amplified with forward primer (5′-CATCATTTTGGCAAAGCCACCATGTCGCTGACTGGTGCCC-3′) and reverse primer (5′-TTGGCAGAGGGAAAATTACTTATCGTCGTCATCCTTGTAATCCTCCAGAACTTTCGGTGGGA-3′) and was cloned into pCAGGS via *Eco*RI and *Bgl*II digestion, resulting in a recombinant eukaryotic expression plasmid pCAGGS-Flag-NSP7α. The pET-24a-His-NSP7α was transformed into competent *Escherichia coli* BL21(DE3). Protein expression was induced by the addition of β-D-1-thiogalactopyranoside (IPTG) to 1 mM final concentration when the optical density at 600 nm (OD_{600}) reached 0.6. The cells were harvested by centrifugation at 6000*g* at 4 °C for 5 min, resuspended in 20 mL Buffer A (50 mM Tris-HCl, 250 mM NaCl, pH 8.0), and ultrasonically lysed for 15 min on ice. The lysate was centrifuged at 12,000*g* and 4 °C for 30 min. The soluble fraction was filtered through 0.22 μm filters (Millipore, USA). The soluble NSP7α protein was purified by High-Affinity Ni-Charged Resin FF (Genscript, China) and eluted with Buffer A containing gradient imidazole (Solarbio, China).

2.3. SDS-PAGE and Western blotting analysis

NSP7α prokaryotic expression was analyzed by 15 % SDS-PAGE. The resolved proteins were stained with quick staining buffer (Ruibiotech, China). Marc-145 cells were infected with PRRSV HuN4 at a multiplicity of infection (MOI) of 0.1. The cells were harvested at 48 h and lysed in RIPA buffer (Solarbio). BHK-21 cells were transfected with pCAGGS-Flag-NSP7α plasmids. Cell lysates were collected 48 h post-transfection (hpt). For Western blot, individual samples were transferred to PVDF membrane (Millipore) and blocked with 5 % skim milk for 1 h. Membranes were incubated for 1 h with mouse anti-His-tag monoclonal antibody (mAb; Proteintech, USA) or mouse antibody against NSP7α serum was diluted 1:1000 in Tris-buffered saline (TBS) as primary antibody. This was followed by three washes using TBS containing 0.1 % Tween-20 (TBST). Next, each membrane was incubated with a 1:1000 dilution of DyLight 800-labeled antibody against mouse IgG(H + L) (KPL, USA) at room temperature for 1 h. After three washes with TBST, the reactive proteins in the blots were detected using the Odyssey CLx Image Studio (LI-COR, USA).

2.4. Production of antibodies against PRRSV NSP7α

Purified NSP7α protein was diluted to a final concentration of 2 mg/mL in PBS, and mixed with an equal volume of complete Freund's adjuvant or incomplete Freund's adjuvant (Sigma-Aldrich). Six-week-old mice were immunized with 150 μL emulsified protein per mouse by three intramuscular and subcutaneous injections. Three days after the third immunization, the mice were euthanized and serum was collected. Spleen cells were fused with SP2/0 cells and incubated in DMEM containing 20 % FBS, Hypoxanthine-Aminopterin-Thymidin media supplement (Sigma-Aldrich), and 10 % Hybridoma Feeder (Biodragon, China) at 37 °C. After 10 days, culture supernatants were collected and incubated with primary antibodies for ELISA and immunofluorescence assays (IFA). Positive supernatants corresponding to fused cells were expanded, cultured, and sorted using an MA900 cell sorter (Sony, Japan) to obtain monoclonal cells. These cells were dispensed into 96-well plates and cultured in DMEM containing 20 % FBS, hypoxanthine-thymidine (Sigma-Aldrich), and a 5 % Hybridoma Feeder.

2.5. Indirect ELISA

Purified recombinant NSP7α protein was diluted in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6) to a final concentration of 10 μg/mL. One hundred microliters of the protein solution were added to each well of a 96-well ELISA plate (Corning, USA) and left overnight. Coated ELISA plates were blocked with 5 % skim milk in PBST for 1 h at 37 °C. Pig serum samples were diluted 25-fold with PBS, and mouse serum was serially diluted. Then, each well was incubated with 100 μL serially diluted antiserum or mouse anti-His mAb as positive control or PBST as negative control at 37 °C for 1 h. After three washes with PBST, each well received 100 μL of horseradish peroxidase (HRP) conjugated goat anti-mouse antibody (Abcam, USA) or HRP conjugated rabbit anti-pig antibody (Merk, USA) at 37 °C for 1 h followed by three washes. Next, 100 μL of 3, 3′, 5, 5′-tetramethylbenzidine (TMB) solution was added to each well and incubated in the dark at room temperature for 15 min. The reaction was stopped by adding 100 μL of 0.1 M HCl solution. Optical density was measured at 450 nm using an ELx808 microplate reader (BioTek, USA).

2.6. IFA

BHK-21 cells were cultured in DMEM containing 10 % FBS until they reached 90 % confluence. pCAGGS-Flag-NSP7α or pCAGGS empty vector was then transfected into cells and incubation was continued at 37 °C in an atmosphere of 5 % CO_2. Marc-145 cells were cultured to 90 % confluence and then infected with PRRSV HuN4 at a MOI of 0.1. At the indicated time points, cells were harvested, fixed with 4 % paraformaldehyde at 37 °C for 1 h, permeabilized with 0.25 % Triton-X 100 in PBS, and blocked with 5 % bovine serum albumin for 1 h at room temperature. After three washes with PBST, cells were incubated with rabbit anti-Flag or mouse anti-NSP7α or monoclonal cell culture medium supernatant at 37 °C for 1 h. Cells were treated with Cy3 goat anti-rabbit IgG (H + L) (Abclonal, China) and goat anti-mouse IgG (whole molecule)-fluorescein isothiocyanate antibody (Sigma-Aldrich) at 37 °C for 1 h followed by 4′6-diamidino-2-phenylindole (DAPI) (Solarbio). Images were captured using a fluorescence microscope (Thermo Fisher Scientific, USA).

2.7. Epitope mapping

NSP7α truncated fragments were amplified (NSP7α-A and NSP7α-B) or annealed using oligonucleotides. The mCherry-linker fragment was amplified and the GGGGSGGGG linker sequence was introduced at the C terminus using the reverse primer mCherry-R. The NSP7α truncated fragments and mCherry-linker were cloned into the pCAGGS vector using the MonClone™ Hi-Fusion Cloning Mix V2 (Monad, China). For

site mutant NSP7α epitope expression plasmids construction, full-length pCAGGS-mCherry-NSP7α-EP10 mutation plasmids were amplified by using mutation primers, and the template plasmid pCAGGS-mCherry-NSP7α-EP10 was digested by *Dpn*I enzyme. Then, PCR product was transfected into DH5α. All primer sequences are listed in Table S1.BHK-21 cells were seeded in wells of 24-well plates and transfected with truncated NSP7α expression plasmids when they had grown to 90 % confluence. At 36 h post-transfection, cells were fixed, stained with NSP7α mAbs at 37 °C for 1 h, and treated with DAPI for 15 min at room temperature. Fluorescence images were acquired.

2.8. Sequence alignment and structure analysis of NSP7α

NSP7α protein sequences of representative PRRSV strains (NADC30 strain of L1, QYYZ strain of L3, EDRD-1 strain of L4, VR2332 strain of L5, P129 strain of L6, SP strain of L7, CH-1a strain of L8, HuN4 strain of L8, NC16845 strain of L9) were aligned using Geneious software. The locations of the epitopes identified in the present study were predicted using the IDEB website (http://tools.iedb.org/bcell/).

2.9. Statistical analysis

The significance of the observed differences was assessed using a two-tailed, unpaired, and unequal variant of Student's *t*-test with GraphPad Prism 8.0. *P*-values<0.05 were considered significant.

3. Results

3.1. Preparation of PRRSV NSP7α protein

NSP7α was cloned into a pET24a(−) vector containing His-tag at the C terminus and expressed in *E. coli*. BL21(DE3). The NSP7α-His protein recovered from the supernatant had a molecular weight of approximately 15 kDa (Fig. 1A). After affinity purification, the purified proteins were analyzed using SDS-PAGE and Western blotting. A single protein band was detected by SDS-PAGE (Fig. 1B). Western blot analysis indicated that NSP7α was successfully expressed and purified (Fig. 1C).

3.2. Production of anti-NPS7α polyclonal antibody and mAb

To verify the production of the anti-NSP7α antibody, serum samples were serially diluted and used as primary antibody for ELISA analyses. All diluted mouse sera (1:25600) exhibited a positive/negative ratio > 20. The specificity of anti-NSP7α serum was verified in pCAGGS-Flag-NSP7α-transfected BHK-21 cells. Western blotting analysis using anti-NSP7α serum also revealed a specific band as the primary antibody (Fig. S1). These results indicate that immunized mice produced high levels of antibody against NSP7α. Three strains mAbs were isolated (4H9, 3F2 and C10). Marc-145 cells infected with PRRSV HuN4 strain were harvested and analyzed by IFA. A positive signal was detected in PRRSV-infected cells at 36 hpi, but not in mock cells (Fig. 2A), indicating that mAbs against NSP7α could recognize PRRSV-infected cells. Western blotting analysis of NSP7α in PRRSV-infected cells revealed specifical bands with molecular weights of approximately 16, 30, 36, 39, and 110 kDa (Fig. 2C).

3.3. Epitope mapping using NSP7α mAbs

A series of truncated NSP7α eukaryotic expression plasmids were constructed (Fig. 3A). These plasmids were transfected into BHK-21 cells, and identified by IFA with NSP7α mAbs as primary antibody. First, we evaluated the ability of mAbs to recognize NSP7α-A and NSP7α-B. All three mAbs (4H9, 3F2, and C10) recognized NSP7α-B (amino acids (aa) 72–149 of NSP7α) (Fig. 3B), but not aa 1–89. NSP7α-B was further truncated into 10 polypeptides and inserted into the pCAGGS vector. The 4H9 mAb only recognized aa 135–149 and did not recognize the other nine polypeptides (Fig. 3C). Unexpectedly, 3F2 and C10 mAbs did not recognize any of the 10 polypeptides. To further verify the amino acid recognized by 4H9 mAb, the 4H9 mAb could reacted with NSP7α-EP10m1 and NSP7α-EP10m2. The aa 144–146 and aa 147–149 of NPS7α were mutated to alanine do not affect the reactivity of 4H9 mAb (Fig. 3D). This result demonstrates that aa 135–143 is critical for 4H9 mAb binding. 137th or 140th residue of NSP7α was mutated to alanine, the mutant EP10 could be recognized by 4H9 mAbs (Fig. 3E). Sequence alignment of representative strains of different lineages of PRRSV-2 showed that NSP7α is highly conserved in aa 135–143 (Fig. 4A), except for the substitution of two aa. The mAb recognition region was analyzed; a region from aa 135–143 was located on the

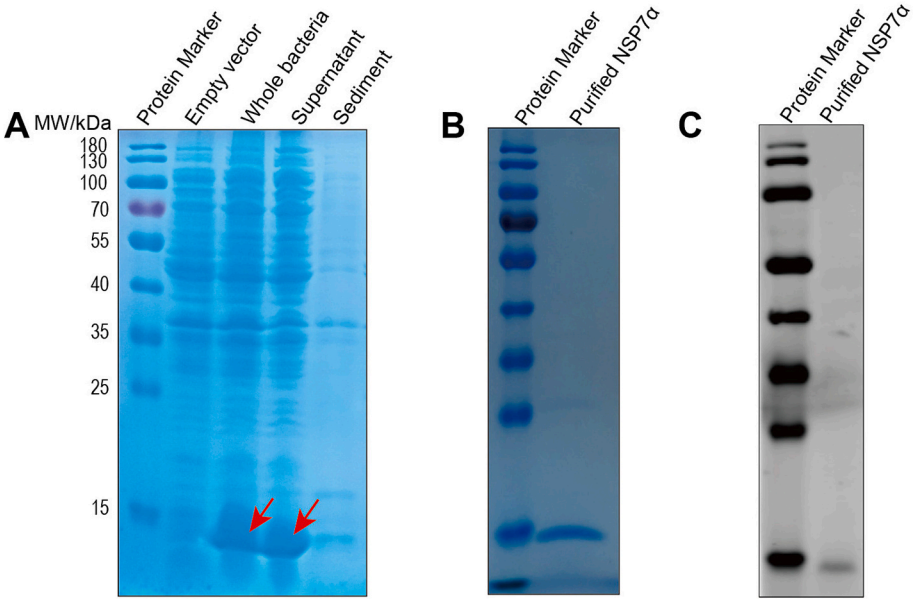

Fig. 1. Expression and purification of NSP7α. (A) SDS-PAGE analysis of NSP7α expression in *E. coli*. NSP7α is indicated by red arrows. Purified NSP7α was identified by SDS-PAGE (B) and Western blot (C).

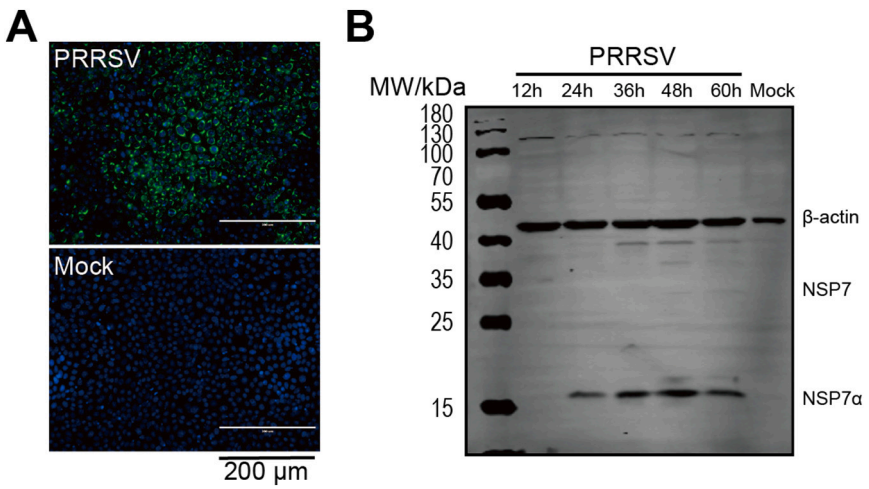

Fig. 2. Reactivity of strain 4H9 mAb with PRRSV NSP7α. (A) Marc-145 cells were infected with HuN4 at an MOI of 0.1. At the indicated time points, cells were harvested and analyzed by IFA (A) (36 hpi) and Western blotting (12, 24, 36, 48, and 60 hpi) (B).

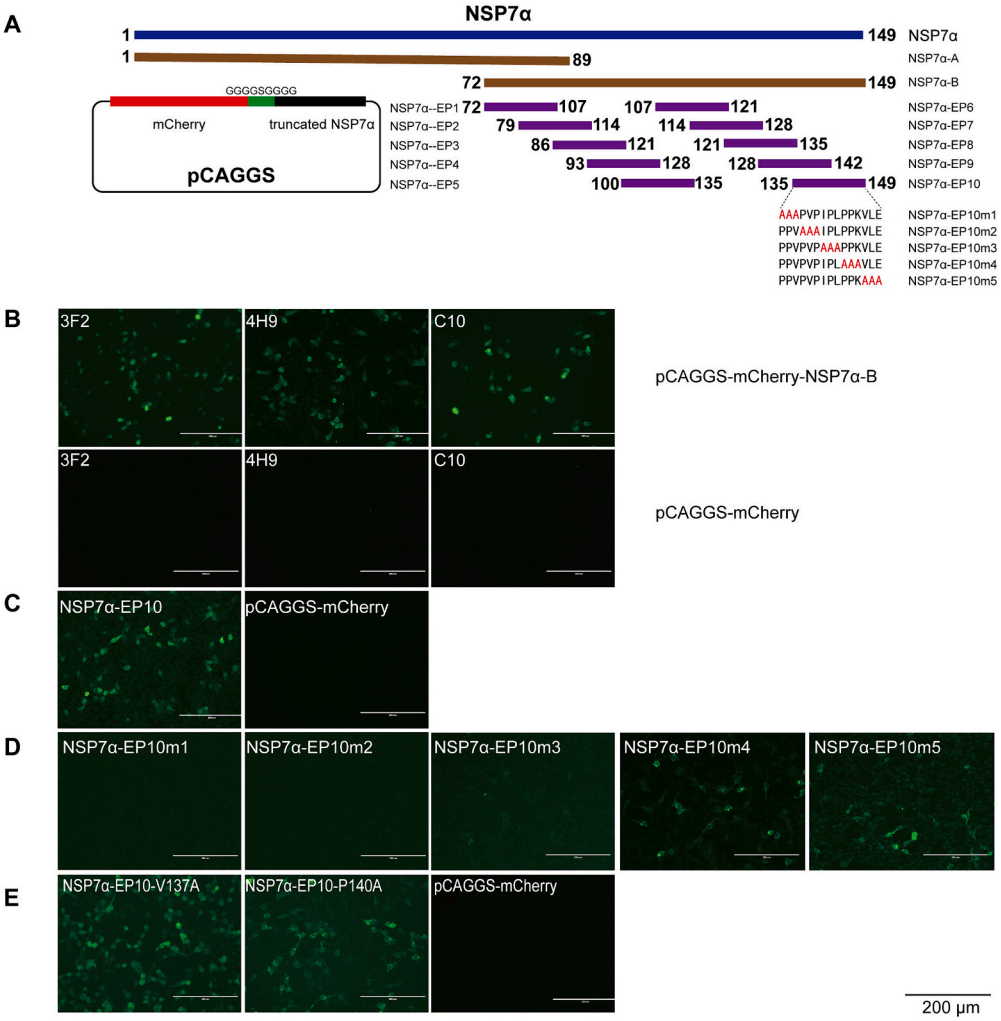

Fig. 3. Epitope mapping of NSP7α mAbs. (A) Schematic drawing of NSP7α fragments and eukaryotic expression plasmid. (B, C, D & E) BHK-21 cells were transfected with truncated NSP7α expression plasmids. At 24 hpi, cells were fixed and stained with NSP7α mAbs. The fluorescence images were acquired by inverted fluorescence microscopy.

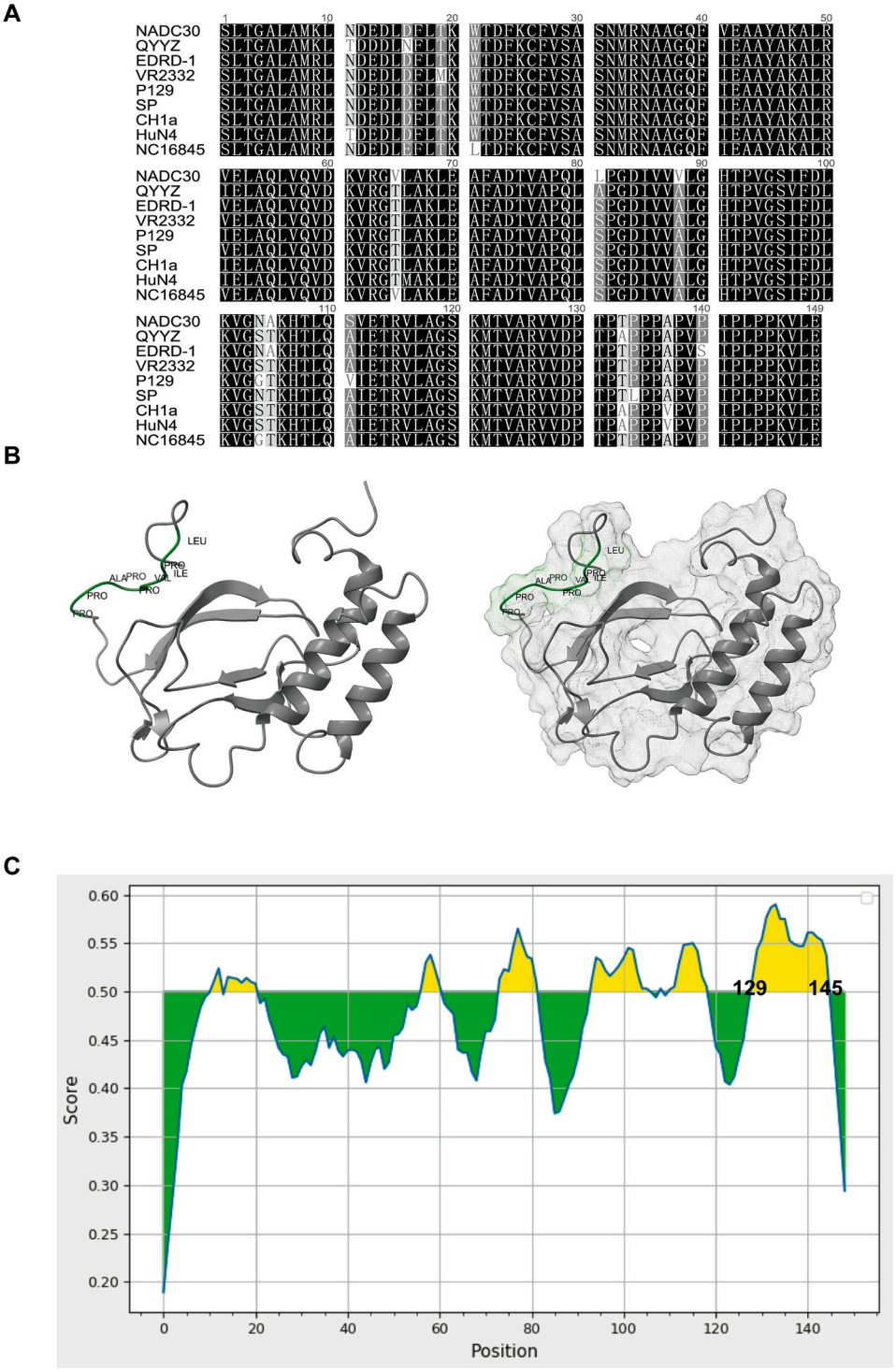

Fig. 4. Multiple sequence alignment and structural analysis of NSP7α. (A) NSP7α amino acid sequences of different lineage representative strains were aligned using Geneious software. (B) The detailed position of aa 135–143 of NSP7α was analyzed. (C) The B cell linear epitope of NSP7α was predicted by IEDB analysis resource. Yellow indicates the epitope residues.

surface of NSP7α (Fig. 4B). The antigenicity of NSP7α was predicted by the IEDB website; aa 129–145 displayed high antigenicity (Fig. 4C), which is consistent with the IFA results.

3.4. Reactivity of NSP7α mAbs with PRRSV-1 and different lineage strains of PRRSV-2

To verify the reactivity of NSP7α mAbs with different lineages of PRRSV, Marc-145 cells were infected with four representative lineages of PRRSV-2 and one representative PRRSV-1. The mAbs of NSP7α were probed by IFA. The 4H9, 3F2, and C10 mAbs reacted with the PRRSV-2 L1, L3, L5, and L8 strains, but not with PRRSV-1 (Fig. 5).

3.5. Detection of NSP7α in PRRSV-infected cells

To detect the expression of NSP7α in PRRSV-infected cells, Marc-145

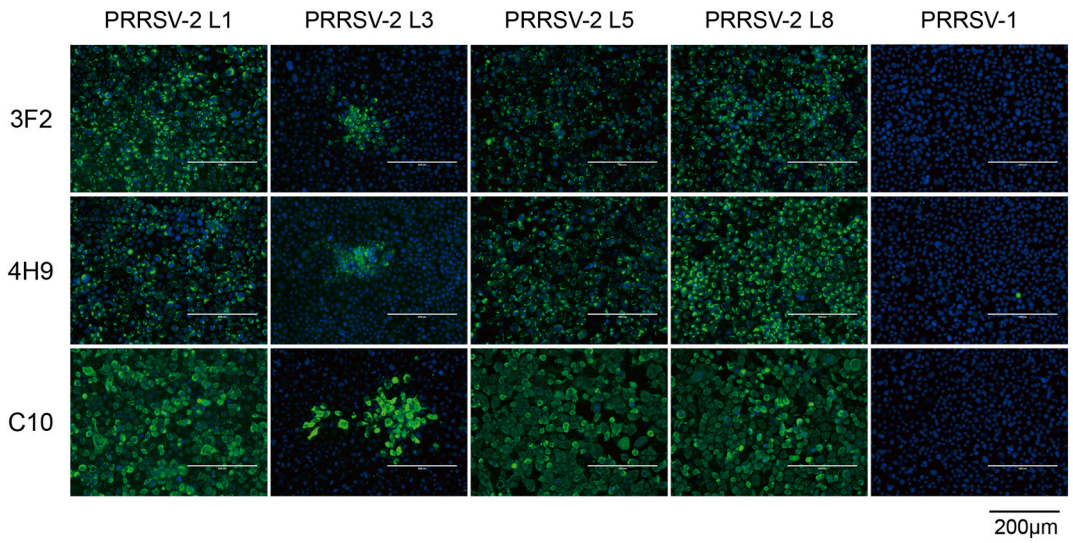

Fig. 5. Reactivity of mAbs with different lineage strains of PRRSV. Marc-145 cells were infected with different strains (S043, GD59, lngelvac MLV, and HuN4) of PRRSV-2 or PRRSV-1. At 48 hpi, cells were fixed and stained with NSP7α mAbs. Images were acquired by inverted fluorescence microscopy.

cells were infected with PRRSV. At different time points (4, 8, 12, 24, and 36 h), cells were fixed and stained with NSP7α or N mAb. At 4 hpi no positive signal was detected for either N or NSP7α. NSP7α and N were detected from 8 hpi onward. NSP7α was localized in the cytoplasm as aggregates around the nucleus. N protein was uniformly localized in the cytoplasm and nucleolus (Fig. 6).

3.6. Antibody response of NSP7α in PRRSV-infected piglets and clinical pig sera

The purified NSP7α was coated with different concentrations, PRRSV positive pig serum was diluted in different ratios as the primary antibody. The optimal coating concentration was determined to be 10 μg/mL, and the optimal serum dilution was 1:25. NSP7α antibody of 131 clinical sera were detected by NSP7α-ab-ELISA and compared with IDEXX PRRS X3 ab test kit. Of the 90 IDEXX PPRSV X3 test positive samples, 83 of sera were positive for NSP7α antibodies. Of the 41 IDEXX PPRSV X3 test negative samples, 39 of sera were negative for NSP7α antibodies (Table. 1). Sera collected from PRRSV-infected piglets was analyzed by NSP7α-coated indirect ELISA. NSP7α specific antibody was detectable at 14 dpi in all infected piglets (Fig. 7). The Sample/Positive ratio > 0.573 is considered antibody positive; the results indicated the NSP7α protein was immunodominant in PRRSV-infected piglets.

4. Discussion

PRRS has caused significant economic losses to the global pig industry since its emergence in 1987. Prevention and control depend mainly on vaccinations. PRRSV vaccines, including inactivated and live-attenuated vaccines, are widely used [11]. Diagnostic methods for NSP can be used to evaluate immunization efficiency and distinguish between wild-type virus infection and vaccine immunization. As one of the most conserved nonstructural proteins of PRRSV, NSP7 plays an important role in PRRSV replication [3]. The strong and continuous antibody response specific to NSP7 can be detected as early as 14– dpi, and the antibody titer can be higher than that of the structural protein N antibody [12]. A fluorescent microsphere immunoassay with good sensitivity and specificity was developed for the detection of NSP7 antibodies in oral fluid samples [13]. In this study, we established an indirect ELISA diagnostic method and detected the NSP7α antibody of sera which from artificially infected samples and clinical samples. NSP7α antibody could be detected at 14 dpi. The NSP7α-ab-ELISA has 93.1 % coincidence with IDEXX PRRS X3 ab test kit, some sera judged to be positive by IDEXX test kit were negative by NSP7α-ab-ELISA, this may be because these samples were from early stage of PRRSV infected pig. These results suggest that NSP7α antibody is good potential detection target to PRRSV infection diagnosis. Because of the good antigenicity of NSP7, detection of the NSP7 antibody could be used to develop differential diagnostic methods to distinguish sera after immunization with inactivated or attenuated vaccines. Identification of the NSP7 epitope is important for the development of better diagnostic methods.

In this study, PRRSV-2 NSP7α was expressed in *E. coli* and purified for immunization. Three monoclonal antibodies (mAbs) were produced against PRRSV-2 and NSP7. All three identified aa 72–149 as the recognition region. Western blotting analysis results of NSP7α of PRRSV-infected cells revealed protein bands with approximate molecular weights of 16 and 30 kDa representing NSP7α and NSP7, respectively. The alignment results of the PRRSV-2 NSP7 aa sequences showed that different lineage strains of PRRSV NSP7 were highly conserved. Except for the substitution of several aa for NSP7α, aa 135–143 are highly conservative among all PRRSV-2 strains. However, these substitutions did not affect the reactivity of the mAbs. The three mAbs reacted with different lineages of PRRSV-2, and four strains of PRRSV-2 (L1, L3, L5, and L8) reacted with three mAbs (4H9, 3F2, and C10). Although there are replacements at position 137th and 140th of NSP7α, single site mutation of NSP7α aa 135–143 do not affect 4H9 mAbs recognize the polypeptide. The findings indicate that the NSP7α mAbs could be used to develop detection methods. Given the conservation of epitopes recognized by 4H9 mAb, this mAb may be useful for the production of diagnostic reagents for IFA or IHC. The epitope recognized by 4H9 is a proline-rich region adjacent to the cleavage site of NSP7α and NSP7β. Proline-rich regions are present in several viral proteins and play important roles in viral replication, assembly, and secretion [14–16]. Although the proline-rich region of NSP7α has not been researched, it may play an important role in PRRSV replication. Unlike the N protein, which is distributed uniformly in the cytoplasm, NSP7α is clustered around the nucleus, consistent with the involvement of NSP7α in the assembly of PRRSV RTC.

In summary, the antigenicity and epitope mapping of NSP7α of PRRSV-2 was performed and we identified a novel immunodominant epitope, ^{135}PPVPVPIPL^{143}. This epitope is highly conserved among nine PRRSV-2 lineages and single site mutation of 137th and 140th of NSP7α did not affect reactivity of the epitope. The effect of the NSP7 epitope on PRRSV proliferation is worthy of further investigation and may provide reference data for the development of detection methods using NSP7α antibody and negative-labeled vaccines. These results improve our

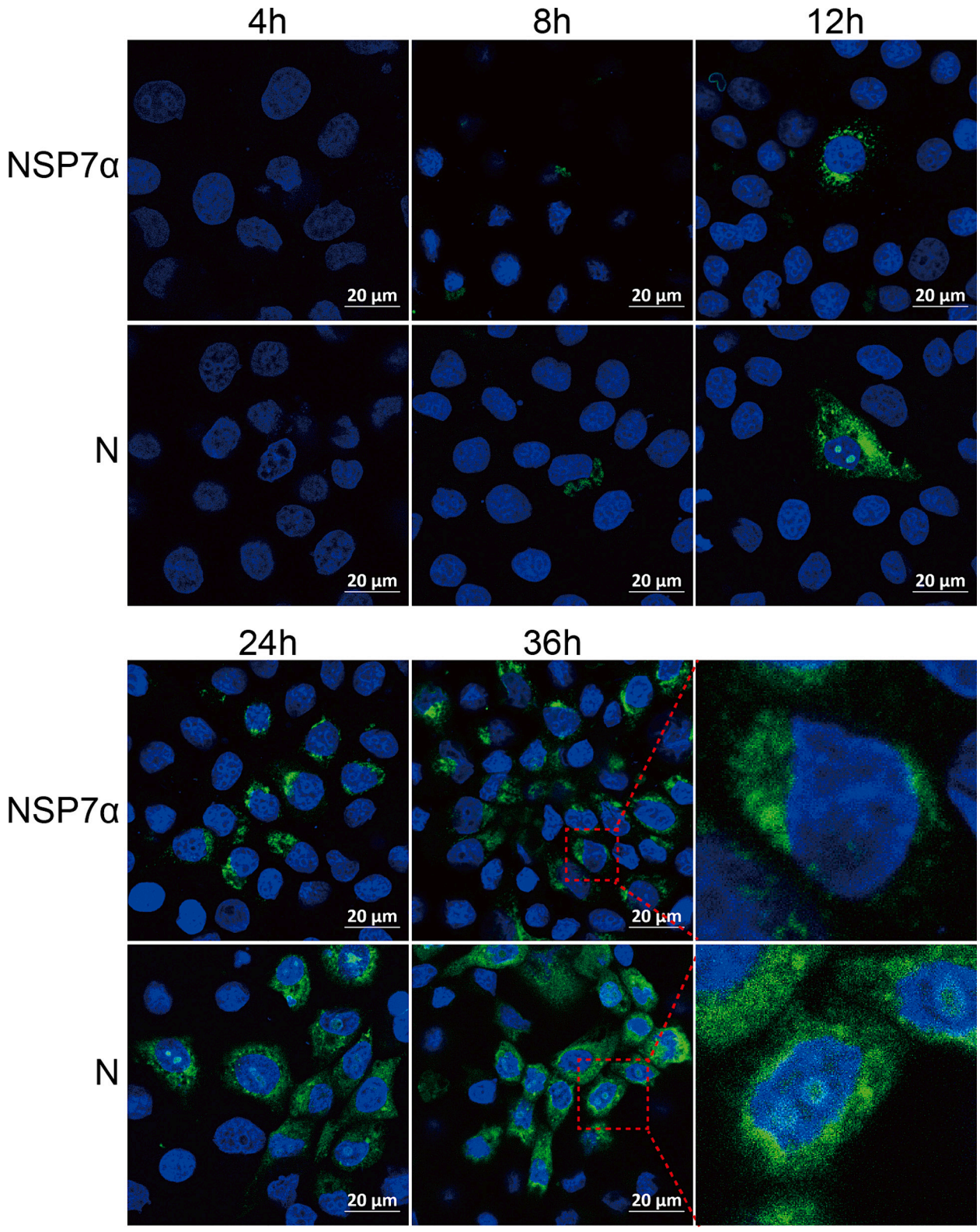

Fig. 6. Detection of NSP7α distribution in PRRSV infected cells. Marc-145 cells were infected with PRRSV HuN4 strain at an MOI of 0.1. At different time points (4, 8, 12, 24, and 36 hpi), cells were fixed and stained with 4H9 strain mAb and visualized by laser confocal microscopy.

understanding of the antigenic structure of PRRSV NSP7 and lay the foundation for developing diagnostic methods.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2024.130944.

Ethics approval

The animal experiment was approved by the Ethics Committee of Harbin Veterinary Research Institute, Chinese Academy of Agricultural Science (approval ID: 210608-09).

Table 1
Comparison of detection results between IDEXX kit and NSP7α-ab-ELISA for clinical sera.

	NSP7α-ab-ELISA	IDEXX	Coincidence Rate
Positive	85(83*)	90	92.2 %
Negative	46(39*)	41	95.1 %
Total	131(122*)	131	93.1 %

Note: The number marked with "*" in brackets indicates the number of samples with consistent detection results between the two methods.

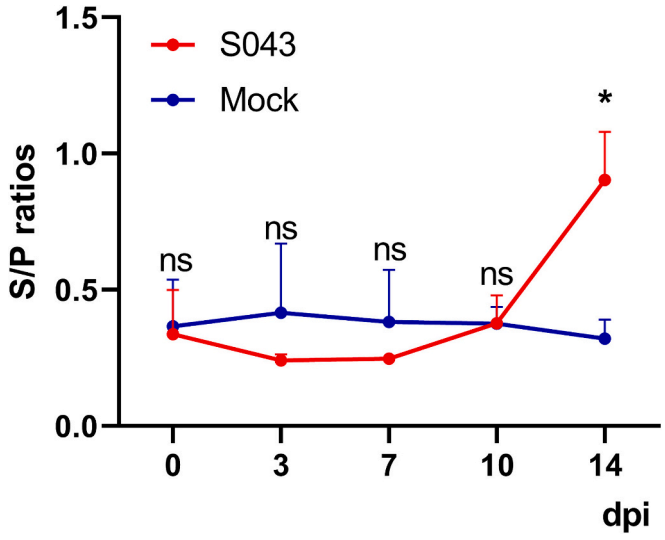

Fig. 7. NSP7α antibody response of PRRSV-infected piglets. Four-week-old piglets were infected with S043 strain of PRRSV-2. NSP7α antibody was detected by ELISA with 25-fold diluted serum as primary antibody. ns: no significant difference, *: $p < 0.05$.

CRediT authorship contribution statement

Tao Wang: Writing – review & editing, Visualization, Validation, Investigation, Formal analysis, Conceptualization. **Da-Song Xia:** Investigation. **Xiao-Xiao Tian:** Investigation. **Yong-Bo Yang:** Writing – review & editing, Resources, Conceptualization. **Tong-Qing An:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Tongqing An reports financial support was provided by the Heilongjiang Provincial Natural Science Foundation of China. Tongqing An reports financial support was provided by Heilongjiang Key Research and Development Program. Tongqing An reports financial support was provided by the National Natural Science Foundation of China.

Data availability

Data will be made available on request.

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