Cloning, expression and monoclonal antibody of porcine interferon-regulated antiviral gene

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Abstract

Background: IRAV (interferon-regulated antiviral gene) was identified with antiviral activity as a novel interferon-stimulated gene. IRAV is upregulated in response to type I and type II IFNs and a number of virus. However, the antiviral activity of IRAV to virus infection is poorly understood.

Results: In this study, we cloned the full-length IRAV complementary DNA (cDNA) from porcine kidney cells. The porcine IRAV cDNA was of 1241 bp with an open reading frame of 858 bp, encoding a polypeptide of 285 amino acids, which localized to the cytoplasm. The porcine IRAV protein was expressed in Escherichia coli BL21 (DE3), purified and immunized to female BALB/c mice to get monoclonal antibodies (MAbs) against porcine IRAV. Five strains of hybridoma cells named 2B10, 2G12, 2H1,5A8 and 2C5 secreting anti-IRAV MAbs were obtained. By western blot analysis and indirect immunofluorescence assay, the MAbs were identified with the specific reaction with the overexpressed porcine IRAV protein in PK15 cells.

Conclusions: The MAbs against porcine IRAV, identified by western blot and IFA, provid a valuable tool to study the biological function of IRAV in the future.

Background

Interferon-stimulated genes (ISGs) mediating the antiviral effect of IFN can block virus at multiple steps including viral entry, translation initiation, propagation, and spread (1; 2). IRAV (Also known as SFL; UPF0515; RyDEN; C19orf66) is identified as a novel interferon-stimulated gene, which inhibits Dengue virus replication (3; 4). IRAV was firstly identified as one of genes upregulated in Daudi cells treated by IFN (5). The published microarray data shows that IRAV is upregulated in response to type I and type II IFNs (5–7) and upregulated in response to a number of virus, including yellow fever virus (8), adenovirus (9), influenza virus (10), Lassa virus (11), ebola virus (12), Marburg viruses (12), human herpesvirus 1 and human herpesvirus 8 (13; 14).

Swine are an important source of proteins worldwide but are subject to frequent viral outbreaks (15): porcine epidemic diarrhea virus (PEDV), porcine reproductive and respiratory syndrome virus (PRRSV), pseudorabies virus (PRV) (16) and african swine fever virus (ASFV) (17). From August 2018, ASFV was outbreak in China (17), and cause a huge economic loss in pig farming. The extensive antiviral effect of IRAV suggests its potential in protecting pig from viral disease. However, little is known on porcine IRAV. In the present work, we cloned the full porcine IRAV gene, expressed and purified the porcine IRAV protein. To facilitate the study of the antiviral activity of IRAV to virus infection, we used the recombinant porcine IRAV protein (rpIRAV protein) to prepare monoclonal antibodies (MAbs) against porcine IRAV, which are the preferred antibodies for investigating the functions of IRAV and helpful in breeding pigs with resistance to viral diseases.

Methods

Cells, antibodies and animals

SP2/0 myeloma cells, porcine kidney cells (PK-15) and African green monkey kidney cells (Vero E6) were obtained from the Shanghai Veterinary Research Institute (CAAS, Beijing, China). Dulbecco modified Eagle's medium (DMEM; Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY) was used to culture all cell lines at 37°C in a humidified 5% CO_2 incubator. Opti-MEM cell culture medium was purchased from Gibco (Grand Island, NY). Anti-FLAG antibody anti-β-actin antibody were purchased from Sigma (Shanghai, China). Alexa Fluor 488 donkey anti-mouse IgG (H+L) antibody and horseradish per-oxidase (HRP)-conjugated goat anti-mouse were purchased from Sigma (Shanghai, China). The 6-week-old female BALB/c mice were purchased from the Shanghai Slack Laboratory Animal (Shanghai, China).

Cloning and sequence analysis of porcine IRAV

Total RNA of swine was isolated from porcine spleen tissue using the TRIzol (Invitrogen). The SuperScript III Reverse Transcriptase was used to synthesize cDNA fragments from extracted total RNA with oligo(dT) primer according to the manufacturer's instructions. Based on the porcine mRNA sequence (GenBank ID nos. NM_001244321), the primers (Table 1) were designed to clone the partial IRAV gene by reverse transcriptase polymerase chain reaction (RT-PCR). Rapid amplification of cDNA ends (RACE) was also performed using porcine IRAV-specific primers (Table 1) and pfu DNA polymerase (Stratagene) to amplify the termini of the IRAV transcript according to the manufacturer's instructions (Park et al., 2012). The PCR products were cloned and sequenced.

The open reading frame (ORF) of the porcine IRAV was analyzed by Open Reading Frame Finder (https://www.ncbi.nlm.nih.gov/orffinder/) on NCBI. Phylogenetic tree based on the predicted amino acid sequence of IRAV was constructed in the MEGA 4 program with 1,000 bootstrap replications using the Neighbor-joining method and the P distance algorithm of correction.

Expression vector construction and subcellular localization

The ORF of porcine IRAV was amplified from the cDNAs obtained from the porcine spleen tissue using the primers designed based on the eukaryotic expression vector p3×FLAG CMV 7.1 (Terminal FLAG tag; Sigma-Aldrich) to produce the pFLAG-pIRAV (Table 1), and the prokaryotic expression vector pCold-I to produce the p-Cold-pIRAV (Table 1).

PK-15 cells were plated in 6-well culture plates and transfected at 70–80% confluency with pFLAG-pIRAV in Gibco OPti-MEM cell culture medium (Life Technologies) using FuGENE HD transfection reagent (Promega) according to the manufacturer's instructions. After 48 h transfection, the cells were washed

and fixed with paraformaldehyde. After staining with DAPI, the cells were observed under a confocal immunofluorescence microscope (Carl Zeiss, Oberkochen, Germany).

Purification of rpIRAV protein and immunization procedure of mice

The recombinant plasmid p-Cold-pIRAV was expressed in *Escherichia coli* BL21 (DE3) at 16℃ for 16 hours by the addition of 0.5 mM of isopropyl-β-D-thiogalactoside (IPTG). The induced rpIRAV protein was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis. The recombinant protein was purified by Nickel Magnetic Beads (Biotool, Shanghai, China) after centrifugation and ultrasonication.

With the equal amount of Freund's complete adjuvant, the purified rpIRAV protein was mixed and emulsified to immune 6-week-old female BALB/c mice through subcutaneous injection. After 2 weeks, each mouse was subcutaneously injected using the same dose of antigen emulsified in Freund's incomplete adjuvant at a 1:1 (v/v) ratio. The immunization was repeated twice at 2-week intervals. Before cell fusion, booster immunization was given 3-4 days in advance. After that, mice were euthanized by cervical dislocation and their spleen were removed aseptically based on animal welfare law of China.

Indirect enzyme-linked immunosorbent assay

Indirect ELISA was used to choose the serum and cell culture containing the highest titer of anti-rpIRAV antibodies. The ELISA Plates were plated with 200 ng/well rpIRAV protein diluted with 100 μL carbonate bicarbonate buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$ [pH 9.6]) and coated at 4℃ overnight. Then the plates were blocked with 5% skimmed milk in phosphate buffer with 0.05% Tween-20 (PBST) at 37℃ for 1 hour. Followed by washing for three times, the plates were incubated at 37℃ containing 100 μL diluted cell culture supernatant or antibodies. An hour later, the plates were incubated with HRP-conjugated goat anti-mouse IgG with 1:20,000 dilution in PBST at 37℃ for 1 hour after washing thrice with PBST. And then, away from light, the plates were incubated with 100 μL/well of TMB liquid for 15 minutes at room temperature. Being stopped by 50 μL/well 2 M H_2SO_4, these plates were read at OD450 value to screen the positive hybridoma cells compared with negative control coating with His-tag protein.

Preparation of anti-rpIRAV protein-specific monoclonal antibody

By indirect ELISA, we determined the mice whose serum contains the highest titer of anti-rpIRAV antibodies. Their spleen cells were fused with SP2/0 myeloma cells under the action of 50% PEG as fusion agents. The hybridoma cells were cultured in hypoxanthine-aminopterin-thymidine (HAT) screening culture medium with 20% FBS in 96-well plates at 37℃ in a humidified 5% CO_2 incubator.

When the cells covered between a third and a half of the bottoms of 96-well plates, we used indirect ELISA to filter the positive hybridomas. The positive hybridoma cells were injected into pristine-treated BALB/c mice after cloning four times by limiting dilution to generate abundant ascetic fluid containing the MAb.

Western blot analysis

Western blot was used to confirm the specificity of the MAbs (18). The monoclonal antibody cell lysate was transferred to the nitrocellulose (NC) membrane after collection and separation through 10% SDS-PAGE. The membrane was blocked for 1 hour at room temperature with 5% skimmed milk on a shaking table in TBST (TBS with 0.1% Polysorbate-20). And then, the NC membrane was incubated with the anti-FLAG antibody, anti-β-actin antibody and anti-IRAV MAbs for 1 hour at room temperature and washed for three times with TBST. After that, the HRP-conjugated goat anti-mouse IgG (1:6000 dilution in TBST) was added on the NC membrane for 1 hour at room temperature. Washing the NC membrane as the method above, we developed color using the SuperSignal West Pico chemiluminescent substrate (Thermo Fisher Scientific, Waltham, MA)

Indirect immunofluorescence assay

Immunofluorescence assays (IFAs) were performed as described previously (19). PK-15 cells were plated in a six-well plate. After 80%confluency, the cells were fixed by paraformaldehyde for 30 minutes at room temperature. Then washing by PBS for three times, cells were blocked with 10% bovine serum albumin in PBS for 1 hour at 37℃. The cells were incubated with the anti-IRAV or anti-FLAG monoclonal antibody (MAb) in PBS at 37℃ for 1 hour after washing by PBS. Followed by washing in PBS, the cells were incubated in a 1:800 dilution of Alexa Fluor 488-labeled goat anti-mouse IgG (H+L) antibody (Invitrogen) for 1 hour. After the final washing step, cells were visually analyzed using fluorescence microscope.

Results

Clone and analysis of porcine IRAV gene

The complete sequence of the porcine IRAV transcript was cloned from porcine spleen cDNA by RT-PCR and RACE. The cDNA was synthesized from the total RNA of porcine spleen tissue using SuperScript III Reverse Transcriptase with oligo(dT) primer according to the manufacturer's instructions (20). The RT-PCR specific primers were used to amplify the partial porcine IRAV sequence containing the full ORF of porcine IRAV, and RACE primers designed based on porcine IRAV sequence were used for RACE to clone the termini of the IRAV transcript according to the manufacturer's instructions. The full sequence of the

porcine IRAV transcript was of 1241 bp, excluding the 3' polyadenylated sequence. ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/) on NCBI showed that 858 bp of porcine IRAV ORF encodes a polypeptide of 285 amino acids. The predicted AA sequence of the porcine IRAV compared with reference IRAV AA sequences available on GenBank showed that porcine IRAV shared the highest level of AA sequence identity (96.2%) with the whale IRAV, and shared AA sequence identities of 95.9%, 94.5%, 94.2%, 94.2%, 93.2%, 92.8% 92.1%, and 91.8%, with the canine, bovine, sheep, horse, theropithecus gelada, human, mouse and macaca mulatta IRAV proteins, respectively (21). Phylogenetic analyses indicated that porcine IRAV protein clustered with the IRAV protein of whale, canine, bovine and sheep (Fig. 1) (21).

Subcellular localization

The ORF of porcine IRAV was amplified from the cDNAs, and cloned into p3 × FLAG CMV 7.1 vector to produce the pFLAG-pIRAV. PK-15 cells in 6-well culture plates was transfected at 70–80% confluency with the pFLAG-pIRAV using FuGENE HD transfection reagent (Promega). After 48 h transfection, the cells were washed and fixed with paraformaldehyde, followed by incubating with Anti-FLAG antibody and secondary antibody (Green). Cell nucleus were stained by DAPI (Blue) (20; 21). The fluorescence signals were visualized by confocal immunofluorescence microscopy. IRAV fusion proteins were detected to be distributed predominantly in the cytoplasm of the PK-15 cells (Fig. 2).

Expression and purification of rpIRAV protein

The p-Cold-pIRAV was produced by cloning and inserting porcine IRAV ORF to the prokaryotic expression vector pCold-I, which was confirmed by sequencing (19). The recombinant plasmid p-Cold-pIRAV expressed in Escherichia coli BL21 (DE3) migrated at 35 kDa on SDS-PAGE, which was consistent with the expected molecular weight (Fig. 3). The optimized studies indicated that the highest expression of the recombinant protein was found with conditions of 16℃ and 0.2 mM IPTG for 16 h incubation. In the solubility study, the induced protein was found mainly in the supernatant in E. coli efficiently, and was easily purified by using His-binding Nickel Magnetic Beads (Biotool, Shanghai, China) after centrifugation and ultrasonication (Fig. 3) (19).

Generation of MAbs against porcine IRAV

Five groups of BALB/c mice were immunized with the purified rpIRAV protein to prepare MAbs. Indirect ELISA was used to choose the serum from immunized mice containing the highest titer of anti-rpIRAV antibodies (18). Before cell fusion, booster immunization was given 3–4 days in advance. The spleen cells of mice were fused with SP2/0 myeloma cells to generate hybridoma cell lines expressing MAbs against rpIRAV. After subcloning by limiting dilution and screening for four times, five cell lines secreting positive MAbs were obtained and named 2B10, 2G12, 2H1, 5A8 and 2C5 (Fig. 4A).

Reactivity of MAbs against rpIRAV

To obtain the ascites containing MAbs against rpIRAV, the MAbs 2B10, 2G12, 2H1, 5A8 and 2C5 cell lines were injected to mice (19). After a week, the ascites was extracted from mice, and purified by MAb ProteinG Spin Columns (Thermo Fisher Scientific, Rockford, IL). Followed by overexpressing porcine IRAV

protein in PK-15 cells, the specificity of the MAbs was identified by Western blot analysis and Immunofluorescence assays (IFAs). Western blot analysis showed that the MAbs 2B10, 2G12, 2H1, 5A8 and 2C5 against porcine IRAV protein (Fig. 4A), and IFAs indicated that the MAbs 5A8 against porcine IRAV protein (Fig. 4B).

Discussion

IRAV is an novel IFN-stimulated gene (ISG) with antiviral activity against DENV and HCV (3; 4; 7). The published microarray data shows that IRAV is upregulated in response to type I and type II IFNs (5–7) and upregulated in response to a number of virus, including yellow fever virus (8), adenovirus (9), influenza virus (10), Lassa virus (11), ebola virus (12), Marburg viruses (12), human herpesvirus 1 and human herpesvirus 8 (13; 14). The extensive antiviral effect of IRAV suggests its potential in protecting animal and human from viral disease. Little is known about porcine IRAV. Recently years, PRRSV, PEDV, PRV and ASFV caused a huge economic loss in pig farming. Porcine IRAV protein might be an important molecule in innate antiviral immune responses, as revealed by studies in its antiviral activity (3; 4; 22). Characterization of porcine IRAV and providing its monoclonal antibody will contribute to preventing viral infection and pathogenesis in pigs, and to breeding pigs resistant to viral diseases.

There are several porcine IRAV-like sequences on GenBank, but the sequences do not contain gene annotation. In the present study, porcine IRAV gene and the termini of it was cloned by RT-PCR and RACE from the IRAV mRNA of porcine spleen tissues, which contains an ORF (858 bp) encoding a polypeptide of 285 amino acids that shares the highest level of AA sequence identity (96.2%) with the whale IRAV. Phylogenetic analyses indicated that porcine IRAV protein clustered with the IRAV protein of whale, canine, bovine and sheep (21). Subcellular localization data of porcine IRAV showed that it localized to the cytoplasm.

For the porcine IRAV protein shares low AA sequence identity with human (92.8%), mouse (92.1%) and Macaca mulatta (91.8%) IRAV proteins, it is difficult to detected the endogenous porcine protein using the antibodies of human, mouse and Macaca mulatta IRAV proteins. So, we expressed porcine IRAV gene bacterially to generate the MAbs against porcine IRAV. A high level of antibody induced by the purified rIRAV protein was detected using indirect ELISA in immunized mice. By hybridoma technique, MAbs against porcine IRAV named 2B10, 2G12, 2H1,5A8 and 2C5 were generated. This antibody, identified by Western blot and IFA, provided a valuable tool for further investigation of the antiviral activity of IRAV.

In our current study, the recombinant porcine IRAV protein was expressed and purified for preparing Mabs against porcine IRAV protein. Five cell lines secreting positive MAbs were obtained and named 2B10, 2G12, 2H1, 5A8 and 2C5, the ascites containing MAbs against rpIRAV were obtained, and the specificity of the MAbs was identified by Western blot analysis (Fig. 4A) and Immunofluorescence assays (IFAs) (Fig. 4B). All of their results confirmed the specificity of the MAbs against porcine IRAV protein.

In summary, at the present work, we cloned the full porcine IRAV gene, expressed and purified the porcine IRAV protein, and analyzed the localization of its protein product. To facilitate the study of the antiviral

activity of IRAV to virus infection, we used the recombinant porcine IRAV protein (rpIRAV protein) to prepare monoclonal antibodies (MAbs) against porcine IRAV, which are the preferred antibodies for investigating the functions of porcine IRAV protein and helpful in breeding pigs with resistance to viral diseases.

Conclusion

These preliminary studies lead us to conclude that the monoclonal antibodies (MAbs) against porcine IRAV, are the preferred antibodies for investigating the functions of porcine IRAV protein and helpful in breeding pigs with resistance to viral diseases.

Declarations

Abbreviations

IRAV, interferon-regulated antiviral gene; ISGs, Interferon-stimulated genes; IPTG, isopropyl-β-D-thiogalactoside; PBS, Phosphate-buffered saline; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; HAT, hypoxanthine-aminopterin-thymidine; RT-PCR, reverse transcriptase polymerase chain reaction; HT, medium and hypoxanthine-thymidine; IFA, Indirect immunofluorescence assay; ELISA, Indirect enzyme-linked immunosorbent assay; mAb, monoclonal antibody.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

TS, GT, NK and HW conceived and designed the experiments. HW, YJ, DS, SD, XC, HZ and YB performed the experiments. WT, HZ, HY and LL performed bioinformatic analysis. FG, LY, YJ, YZ, GL and CL provided nutritional advice. TS, GT, HW and NK wrote and revised the manuscript. All authors reviewed the manuscript.

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Availability of data and materials

Data generated and analyzed in this study are presented in this manuscript. Data can be obtained by contacting the corresponding author.

Ethics approval and consent to participate

This study was submitted to and approved by the Ethics and Animal Welfare Committee of Shanghai Veterinary Research Institute, China and Use Committee (Approval No: SHVRI-mo-2018052303). The samples were collected and handled in accordance with the good animal practices required bythe Ethics and Animal Welfare Committee of Shanghai Veterinary Research Institute, China.

Consent for publication

Not applicable.

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References

1.
Sadler AJ, Williams BR. Interferon-inducible antiviral effectors. Nat Rev Immunol. 2008;8:559–68.
2.

Schoggins JW, Rice CM. Interferon-stimulated genes and their antiviral effector functions. Curr Opin Virol. 2011;1:519–25.
3.
Suzuki Y, Chin WX, Han Q, Ichiyama K, Lee CH, Eyo ZW, Ebina H, Takahashi H, Takahashi C, Tan BH, Hishiki T, Ohba K, Matsuyama T, Koyanagi Y, Tan YJ, Sawasaki T, Chu JJ, Vasudevan SG, Sano K, Yamamoto N. Characterization of RyDEN (C19orf66) as an Interferon-Stimulated Cellular Inhibitor against Dengue Virus Replication. PLoS Pathog. 2016;12:e1005357.
4.
Balinsky CA, Schmeisser H, Wells AI, Ganesan S, Jin T, Singh K, Zoon KC. IRAV (FLJ11286), an Interferon-Stimulated Gene with Antiviral Activity against Dengue Virus, Interacts with MOV10. J Virol 91 (2017).
5.
Schmeisser H, Mejido J, Balinsky CA, Morrow AN, Clark CR, Zhao T, Zoon KC. Identification of alpha interferon-induced genes associated with antiviral activity in Daudi cells and characterization of IFIT3 as a novel antiviral gene. J Virol. 2010;84:10671–80.
6.
Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, Rice CM. A diverse range of gene products are effectors of the type I interferon antiviral response. Nature. 2011;472:481–5.
7.
Singh MK, Scott TF, LaFramboise WA, Hu FZ, Post JC, Ehrlich GD. Gene expression changes in peripheral blood mononuclear cells from multiple sclerosis patients undergoing beta-interferon therapy. J Neurol Sci. 2007;258:52–9.
8.
Gaucher D, Therrien R, Kettaf N, Angermann BR, Boucher G, Filali-Mouhim A, Moser JM, Mehta RS, Drake DR 3rd, Castro E, Akondy R, Rinfret A, Yassine-Diab B, Said EA, Chouikh Y, Cameron MJ, Clum R, Kelvin D, Somogyi R, Greller LD, Balderas RS, Wilkinson P, Pantaleo G, Tartaglia J, Haddad EK, Sekaly RP. Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. J Exp Med. 2008;205:3119–31.
9.
Harvey SA, Romanowski EG, Yates KA, Gordon YJ. Adenovirus-directed ocular innate immunity: the role of conjunctival defensin-like chemokines (IP-10, I-TAC) and phagocytic human defensin-alpha. Invest Ophthalmol Vis Sci. 2005;46:3657–65.
10.
Wang J, Nikrad MP, Phang T, Gao B, Alford T, Ito Y, Edeen K, Travanty EA, Kosmider B, Hartshorn K, Mason RJ. Innate immune response to influenza A virus in differentiated human alveolar type II cells. Am J Respir Cell Mol Biol. 2011;45:582–91.
11.
Zapata JC, Carrion R Jr, Patterson JL, Crasta O, Zhang Y, Mani S, Jett M, Poonia B, Djavani M, White DM, Lukashevich IS, Salvato MS. Transcriptome analysis of human peripheral blood mononuclear cells exposed to Lassa virus and to the attenuated Mopeia/Lassa reassortant 29 (ML29), a vaccine candidate. PLoS Negl Trop Dis. 2013;7:e2406.

12.
Kash JC, Muhlberger E, Carter V, Grosch M, Perwitasari O, Proll SC, Thomas MJ, Weber F, Klenk HD, Katze MG. Global suppression of the host antiviral response by Ebola- and Marburgviruses: increased antagonism of the type I interferon response is associated with enhanced virulence. J Virol. 2006;80:3009–20.
13.
Bull TM, Meadows CA, Coldren CD, Moore M, Sotto-Santiago SM, Nana-Sinkam SP, Campbell TB, Geraci MW. Human herpesvirus-8 infection of primary pulmonary microvascular endothelial cells. Am J Respir Cell Mol Biol. 2008;39:706–16.
14.
Miyazaki D, Haruki T, Takeda S, Sasaki S, Yakura K, Terasaka Y, Komatsu N, Yamagami S, Touge H, Touge C, Inoue Y. Herpes simplex virus type 1-induced transcriptional networks of corneal endothelial cells indicate antigen presentation function. Invest Ophthalmol Vis Sci. 2011;52:4282–93.
15.
Shan T, Li L, Simmonds P, Wang C, Moeser A, Delwart E. The fecal virome of pigs on a high-density farm. J Virol. 2011;85:11697–708.
16.
An TQ, Peng JM, Tian ZJ, Zhao HY, Li N, Liu YM, Chen JZ, Leng CL, Sun Y, Chang D, Tong GZ. Pseudorabies virus variant in Bartha-K61-vaccinated pigs, China, 2012. Emerg Infect Dis. 2013;19:1749–55.
17.
Ge S, Li J, Fan X, Liu F, Li L, Wang Q, Ren W, Bao J, Liu C, Wang H, Liu Y, Zhang Y, Xu T, Wu X, Wang Z. Molecular Characterization of African Swine Fever Virus, China, 2018. Emerg Infect Dis. 2018;24:2131–3.
18.
Pan X, Kong N, Shan T, Zheng H, Tong W, Yang S, Li G, Zhou E, Tong G. Monoclonal antibody to N protein of porcine epidemic diarrhea virus. Monoclon Antib Immunodiagn Immunother. 2015;34:51–4.
19.
Kong N, Meng Q, Wu Y, Wang Z, Zuo Y, Tong W, Zheng H, Li G, Yang S, Yu H, Shan T, Zhou EM, Tong G. Monoclonal Antibody to Bone Marrow Stromal Cell Antigen 2 Protein of Swine. Monoclon Antib Immunodiagn Immunother. 2016;35:172–6.
20.
Yang S, Shan T, Zhou Y, Jiang Y, Tong W, Liu F, Wen F, Zhang Q, Tong G. Molecular cloning and characterizations of porcine SAMHD1 and its roles in replication of highly pathogenic porcine reproductive and respiratory syndrome virus. Dev Comp Immunol. 2014;47:234–46.
21.
Shan TL, Tang ZL, Guo DZ, Yang SL, Mu YL, Ma YH, Guan WJ, Li K. Partial molecular cloning, characterization, and analysis of the subcellular localization and expression patterns of the porcine OTUB1 gene. Mol Biol Rep. 2009;36:1573–7.
22.

Yang X, Jing X, Song Y, Zhang C, Liu D. Molecular identification and transcriptional regulation of porcine IFIT2 gene. Mol Biol Rep. 2018;45:433–43.

Table 1

Table 1. Primers used in the present study.

Purpose	Primer names	Sequence (5′-3′)
RT-PCR	IRAV-L1	CCTCCCCCGCCCGAGGTCTG
	IRAV-R1	CACAAAGTATCCACAGACCG
	IRAV-L2	CTCCGCCGCAGGGTGCAGAT
	IRAV-R2	CTGGTTTGCATCTGCACCTG
RACE RT-PCR	RACE-R1	GCCCTCCGTTTCGCTTTCGA
	RACE-R2	TTGGCTCCGCCTCTGCTCTTGC
	RACE-R3	GGCGGGCGCCACCGATCTGC
	RACE-L1	CCCCAAGAGCCGGAAGCAGA
	RACE-L2	AGAACCACCTGCCCAAAGTCC
	RACE-L3	CCCACATCAGCAGTGGCTCC
Expression vector construction	IRAV-P-PCOLD1-L	CGAGGGATCCGAATTCATGTCTCAGGAAGGTGTGGAG
	IRAV-P-PCOLD1-R	CGACAAGCTTGAATTCTCACTCCCCATGCCCAC
	IRAV -FLAG-PL	CGACTCTAGAGGATCCATGTCTCAGGAAGGTGTGGA
	IRAV -FLAG-PR	ATGCCACCCGGGATCCTCACTCCCCATGCCCACCCT

Figures

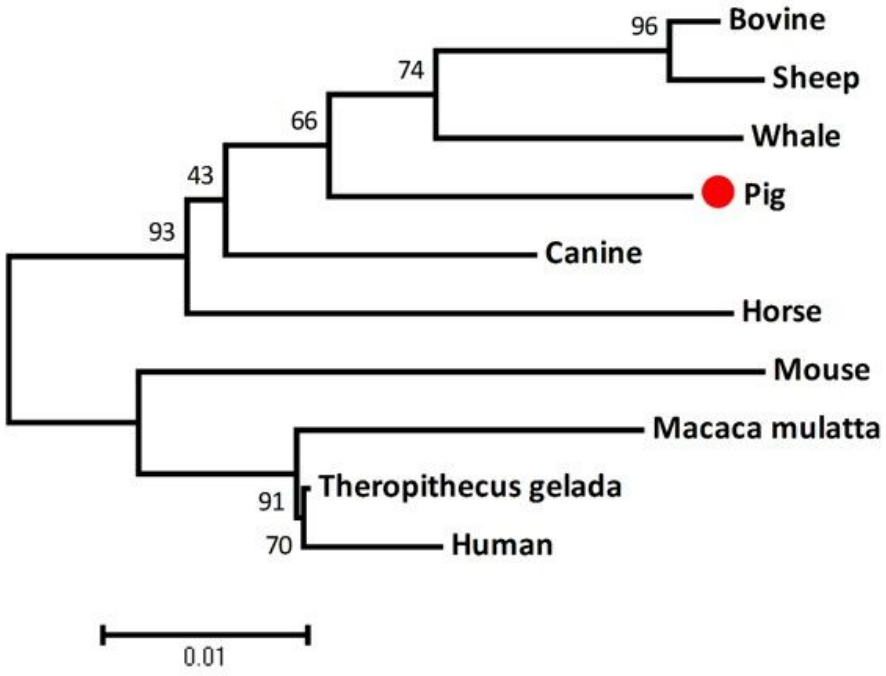

Figure 1

Phylogenetic tree based on the predicted amino acid sequence of full porcine IRAV and reference IRAV AA sequences available on GenBank (whale, canine, bovine, sheep, horse, theropithecus gelada, human, mouse and macaca mulatta) was constructed in the MEGA 4 program with 1,000 bootstrap replications using the Neighbor-joining method and the p-distance algorithm of correction.

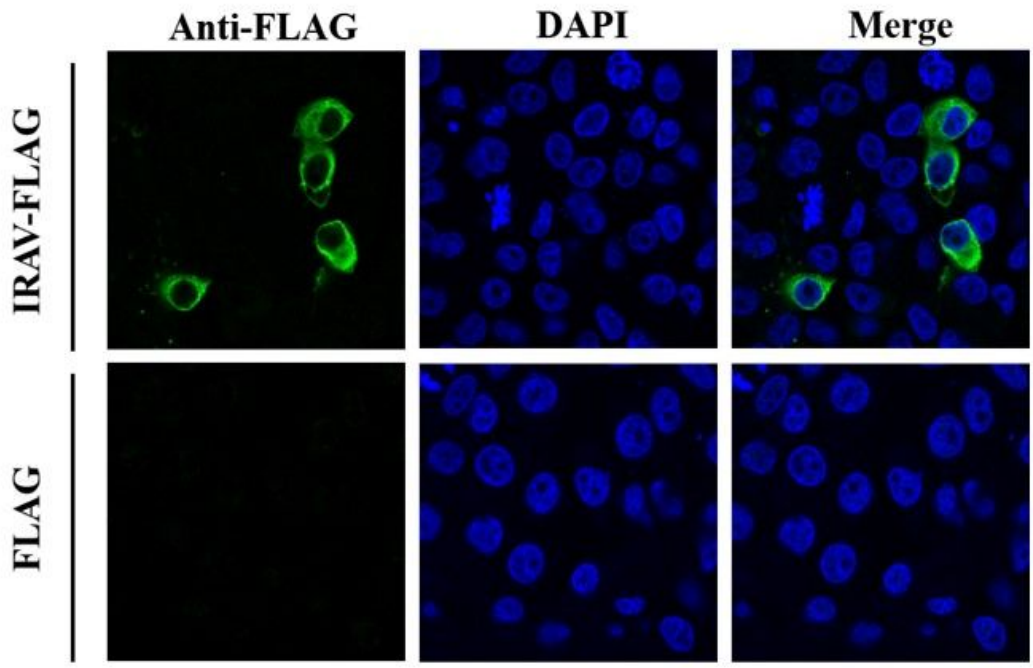

Figure 2

Localization of IRAV in PK-15 cells by Immunofluorescence confocal microscopy analysis. PK-15 cells were transfected with plasmids encoding IRAV-FLAG and FLAG vector for 24 h, followed by incubating with Anti-FLAG antibody and secondary antibody (Green). Cell nucleus were stained by DAPI (Blue). The fluorescence signals were visualized by confocal immunofluorescence microscopy.

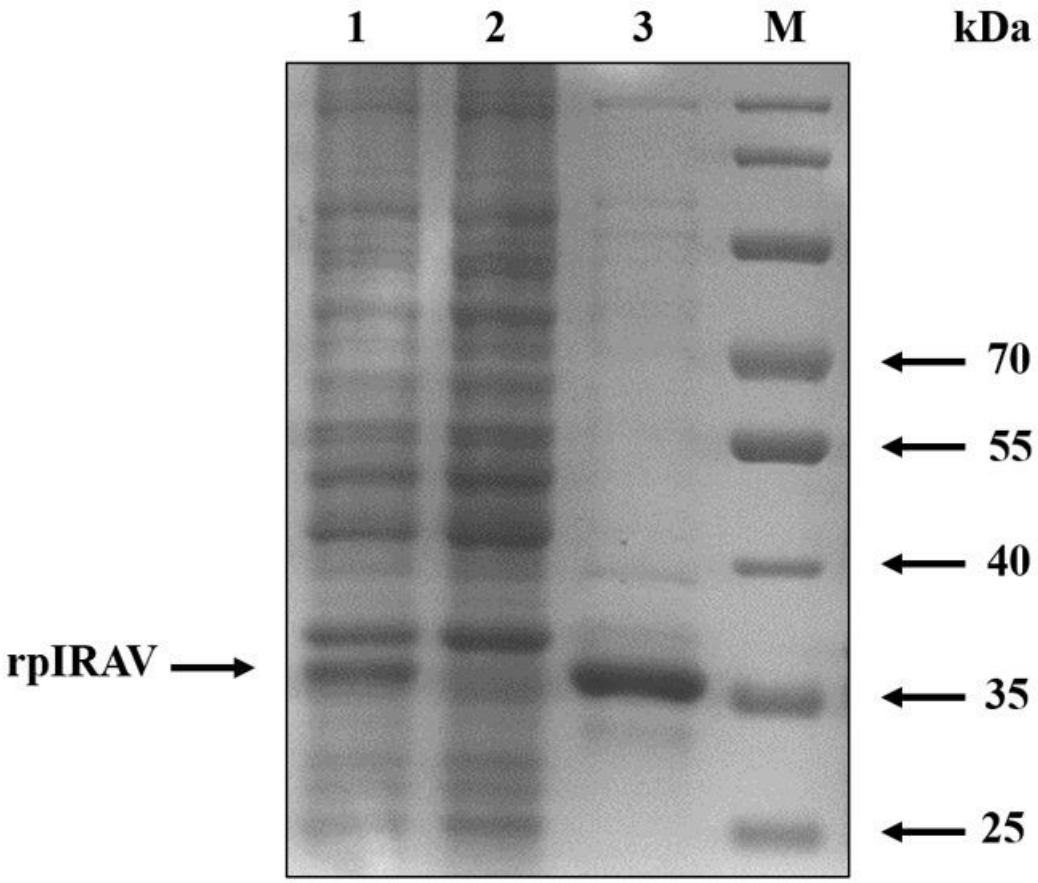

Figure 3

Expression and purification of recombinant porcine IRAV protein analyzed by SDS-PAGE. Lane 1, supernatant proteins; lane 2, precipitation proteins; lane 3, purified rpIRAV protein; M, PageRuler prestained protein ladder. SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

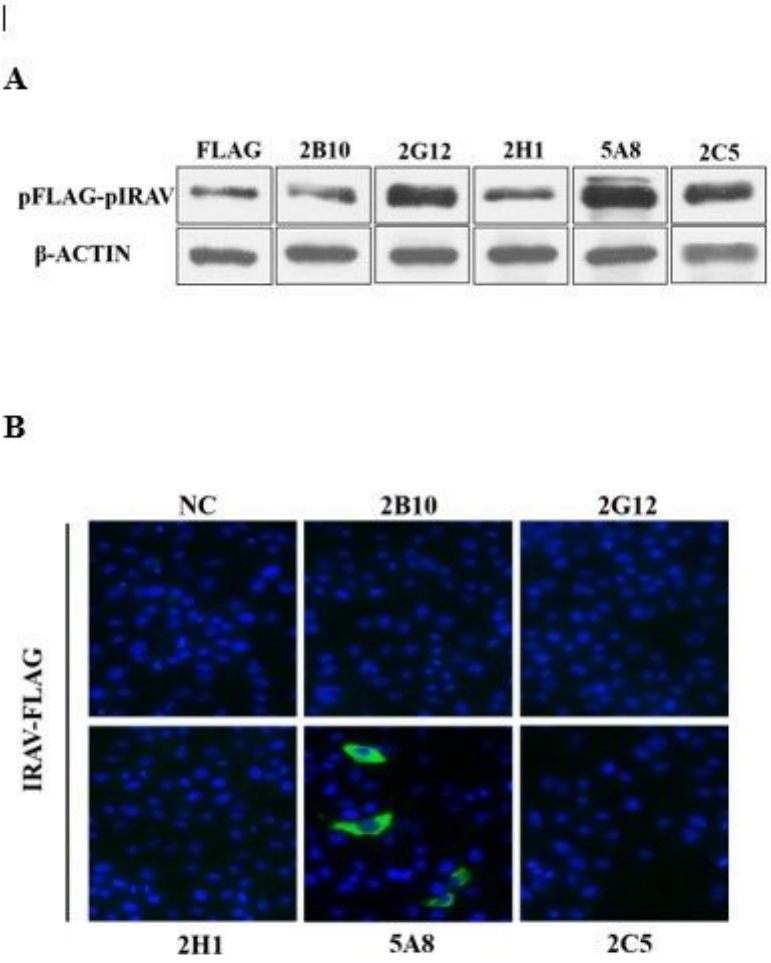

Figure 4

Identification of the SE mAbs. (A) Western blot analyzed the specificity of prepared mAbs against porcine IRAV. Expression of overexpressed porcine IRAV in 293 cells was detected by anti-FLAG antibody, IRAV mAbs 2B10, 2G12, 2H1, 5A8 and 2C5, and anti-β-actin antibody. The protein of β-actin was detected as a control. (B) Indirect immunofluorescence assays analyzed the specificity of prepared mAbs against porcine IRAV. PK-15 cells were plated and transfected in six-well plates. Twenty-four hours later, cells were incubated with normal mouse antibody (negative control) or mab 2B10, 2G12, 2H1, 5A8 and 2C5, followed by Alexa Fluor 488 donkey anti-mouse IgG (H+L) antibody. mAbs, monoclonal antibodies.

Supplementary Files

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