Veterinary Microbiology 158 (2012) 237–246

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Identification of porcine serum proteins modified in response to HP-PRRSV HuN4 infection by two-dimensional differential gel electrophoresis

Yongqian Yang [a], Tongqing An [b], Daqing Gong [b], Dengyun Li [a], Jinmei Peng [b], Chaoliang Leng [b], Zhonghua Yuan [b], Guangzhi Tong [c], Zhijun Tian [b,*], Deli Zhang [a,**]

[a] *Division of Veterinary Microbiology & Virology, College of Veterinary Medicine, Northwest A & F University, Yangling 712100, China*
[b] *Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, China*
[c] *Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai 200241, China*

ARTICLE INFO

Article history:
Received 20 November 2011
Received in revised form 13 January 2012
Accepted 19 January 2012

Keywords:
DIGE
Serum
HP-PRRSV
Inflammation
Acute phase protein

ABSTRACT

Since 2006, highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) has become the major pathogen attributed to the prevalent porcine reproductive and respiratory syndrome (PRRS) in China. The present study aims to identify serum proteins modified in response to infection of HuN4, a HP-PRRSV strain isolated from a farm in 2006. 2-D DIGE analysis allowed for the detection of 19 differentially expressed protein spots, of which 18 were identified by MALDI-TOF/TOF MS. These 18 spots represented for a total of 9 proteins (6 up-regulated and 3 down-regulated), most of which belonged to the acute phase proteins in swine and showed a trend of regression in the late phase of the experiment. One of a series of AGP spots was identified for the first time to be decreased in acute phase of PRRSV infection in swine. But the whole level of the protein in the serum did not show significant changes by Western blot. The rising tendency of Hp was confirmed by Western blot and ELISA. These altered proteins were probably involved in the inflammatory process triggered by HuN4 and in alleviating the oxidative damage occurring in the process. In summary, these results may provide new insights into understanding the mechanisms of HP-PRRSV infection.

© 2012 Published by Elsevier B.V.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of PRRS characterized by reproductive failure in late term gestation in sows and respiratory diseases in pigs of all ages (Lager and Halbur, 1996). In May 2006, a highly pathogenic PRRS (HP-PRRS), characterized by high fever, high morbidity, and high mortality in pigs of all ages, emerged in South China and spread quickly to most of the provinces of China (Li et al., 2007; Tian et al., 2007; Tong et al., 2007). In recent years, highly pathogenic PRRSV (HP-PRRSV) remains extensive pandemic in China (Li et al., 2010; Zhou et al., 2011) and is still evolving quickly (Liu et al., 2011). Researches on the etiology have characterized two discontinuous deletions in NSP2 of the virus (Tian et al., 2007), which was distinguished not to be responsible for the increased virulence of HP-PRRSV (Zhou et al., 2009). The mechanism underlying the molecular pathogenesis of the HP-PRRS remains unknown.

The proteomic approach is promising for investigating the host–virus interaction and has been introduced to research on pig-related virus diseases both in vivo and in

* Corresponding author. Tel.: +86 13946058661/18946066102; fax: +86 13946058661/18946066102.
** Corresponding author. Tel.: +86 13679108785; fax: +86 13679108785.
E-mail addresses: tianzhijun01@yahoo.com.cn (Z. Tian), zhangdeli@tsinghua.org.cn (D. Zhang).

0378-1135/$ – see front matter © 2012 Published by Elsevier B.V.
doi:10.1016/j.vetmic.2012.01.021

vitro (Sun et al., 2010; Zhang et al., 2009). Proteomic analysis of pulmonary alveolar macrophages (PAMs) and lungs following HP-PRRSV infection revealed host responses and changes in protein expression (Xiao et al., 2010; Zhang et al., 2009). In addition, cellular proteins, which may play important roles in viral infectivity, were identified to be incorporated in HP-PRRSV by means of proteomic techniques (Zhang et al., 2010). Compared with the traditional two-dimensional electrophoresis (2-DE), the high throughput two-dimensional difference gel electrophoresis (2-D DIGE) enables multiple protein extracts to be separated on one two-dimensional gel and elimination of gel-to-gel variations by introducing an internal standard (Marouga et al., 2005). Thus, the method has provided a highly sensitive and quantitative tool for analyzing protein changes in various samples.

Serum carries information which may reflect the pathological changes of animals upon virus infection, and provides a promising target for proteomic research on finding potential biomarkers and elaborating the pathogenesis of disease.

To understand more about the pathophysiological response of swine to the newly emerged HP-PRRSV infection, a farrow of piglets were chosen and divided into control and treated/infected groups. We compared the serum protein profiles of piglets infected with HuN4 with those from the normal controls using 2-D DIGE in conjunction with matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry (MALDI-TOF/TOF MS) identification procedures. We also tried to discover possible functions of the differentially expressed proteins involved in the host immunity.

2. Materials and methods

2.1. Experimental animals and virus infection

A farrow of 9 healthy 60-day-old piglets were obtained from a farm in Harbin and randomly divided into control and treated/infected groups. These piglets were free of classical swine fever virus (CSFV), PRRSV, porcine circovirus type 2 (PCV2), porcine parvovirus (PPV) and pseudorabies virus (PRV), confirmed by both serology and PCR at the arrival of the piglets. 5 piglets (Treated 1–5, T1–5) were inoculated with 3×10^3 $TCID_{50}$/mL 5th-passage of HuN4 (Gene Bank accession no. EF635006) intramuscularly (1 mL) and intranasally (2 mL). The rest 4 control piglets (Control 1–4, C1–4) received the same treatment with DMEM. Piglets were kept in separate rooms and monitored 14 days after inoculation. Clinical signs including coughing, dyspnea, anorexia, lameness and shivering were recorded daily. Also, rectal temperature was checked each morning prior to feeding. Blood samples were collected from each pig at 1, 3, 7, 14 days post infection (d.p.i.) in 5 mL glass serum tubes, which were maintained upright at 37 °C for 0.5 h to allow sample coagulation and then 4 °C until they were centrifuged at 2500 rpm at 4 °C for 15 min. Serums were collected into polypropylene tubes, divided into aliquots and snap frozen at −80 °C. The Treated number 4 piglet (T4) died on the 12 d.p.i. Regarding the piglet death and hemolysis status of the samples, 12 samples of 3 piglets (T1–3 and C1–3) at 4 time points were chosen from each group to carry out the DIGE experiment (Table 1). All samples were nicely collected except the sample from Treated 1 on 7 d.p.i. (T1-7d), which showed severe hemolysis.

Table 1
Protein labeling for 2-D DIGE.

Gel	Cy2 (blue)	Cy3 (green)	Cy5 (red)
1	Pool	C1-1d	T1-3d
2	Pool	C2-1d	T2-3d
3	Pool	T3-3d	C3-1d
4	Pool	C1-3d	T2-7d
5	Pool	T1-7d[a]	C2-3d
6	Pool	T3-7d	C3-3d
7	Pool	C2-7d	T3-14d
8	Pool	T2-14d	C1-7d
9	Pool	C3-7d	T1-14d
10	Pool	T1-1d	C1-14d
11	Pool	T2-1d	C2-14d
12	Pool	C3-14d	T3-1d

[a] Sample T1-7d showed severe hemolysis.

2.2. Serological examination

PRRSV-specific antibody was analyzed in all serum samples from the surviving pigs using a commercial HerdCheck PRRS ELISA kit (IDEXX Laboratories Inc., Westbrook, ME, USA) according to the manufacturer's instructions. PRRSV-specific antibody titer was reported as *S*/*P* ratios, and the samples were considered positive if the *S*/*P* ratio was 0.4 or higher.

2.3. Viremia assessment

Viremia assessment was conducted by quantitative RT-PCR technique. A 300-μL volume from each sample was used to extract total RNA according to the directions for the RNeasy Plus Mini kit (Qiagen). A reverse transcription step was performed under the manufacturer's instructions for PrimeScript® RT Reagent Kit (TaKaRa). The reactants were mixed gently and incubated at 37 °C for 15 min and 85 °C for 5 s. The mixture was then examined by *Taq* Man fluorescence quantitative PCR on the Mx3005P Real Time PCR machine according to previous descriptions (Liu et al., 2010).

2.4. Protein labeling for 2-D DIGE

After quantification by the 2-D Quant kit (GE Healthcare), serum samples were prepared in 30 mM Tris, 7 M urea, 2 M thiourea, 2% CHAPS and 1% Protease Inhibitor Mix (GE Healthcare), pH 8.5 buffer to 5–10 μg/μL. Equal amounts of protein from the 24 samples were pooled together as the internal standard. Samples from the control and treated piglets were randomly labeled with Cy3 or Cy5, whereas internal standards were labeled with Cy2 (GE Healthcare) using 400 pmol of fluorochrome (in DMF)/50 μg of protein. Labeling was performed for 30 min on ice in the dark. Reactions were then terminated by the addition of 1 μL of 10 mM lysine for 10 min on ice away from light. 50 μg of control and test samples labeled with

Download English Version:

https://daneshyari.com/en/article/2467094

Download Persian Version:

https://daneshyari.com/article/2467094

Daneshyari.com