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Research paper

Identification of a single nucleotide promoter polymorphism regulating the transcription of ubiquitin specific protease 18 gene related to the resistance to porcine reproductive and respiratory syndrome virus infection

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS), characterized by reproductive failure in sows and respiratory disease and mortality in piglets, is a major infectious disease that causes great economic loss throughout the world. Previous studies revealed that the overexpression of porcine ubiquitin specific protease 18 (*USP18*) gene inhibits PRRSV replication *in vitro*. The objective of this study is to compare the promoter activity of *USP18* in Chinese indigenous Dapulian (DPL) pigs and Duroc × Landrace × Yorkshire (DLY) commercial pigs and screen single nucleotide polymorphism (SNP) affecting porcine *USP18* transcription. We found that the promoter activity was significantly higher in DPL pigs than DLY commercial pigs ($p < 0.05$), deletion of the promoter from −1790 to −1314 bp decreased the transcriptional activity by roughly 60% ($p < 0.05$) and a SNP G–1533A in this region increased the mRNA expression both prior to and post PRRSV infection in MARC-145 cells. Population genetics analysis showed that allele A was only detected in Chinese pig breeds which are generally resistant to PRRSV. These results suggest that the SNP G–1533A polymorphism in the promoter region of porcine *USP18* gene is a potential DNA marker for the resistance to PRRSV.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS), characterized by reproductive failure in sows and respiratory disease and mortality in piglets, is a major infectious disease that causes great economic loss throughout the world (Cho and Dee, 2006). The pathogenic agent, PRRS virus (PRRSV), is a small, enveloped, 15.4 kb, positive-stranded RNA virus that is a member of the *Arteriviridae* family. It predominantly infects the monocyte/macrophage cell lineage of the porcine respiratory system, particularly

Abbreviations: DPL, Dapulian; DLY, Duroc × Landrace × Yorkshire.

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the pulmonary alveolar macrophages (Thanawongnuwech and Thacker, 2003; Zhou and Yang, 2010). The virus displays immune system evasion strategies, and there is considerable antigenic heterogeneity in field virus strains. As a result, current PRRS control strategies, such as vaccination and biosecurity procedures, are not consistently effective in controlling the disease in pig industry. However, accumulating evidence has revealed that breed differences clearly affect the resistance or susceptibility of pigs to PRRSV (Halbur et al., 1998; Lewis et al., 2009; Petry et al., 2005, 2007; Reiner et al., 2010; Vincent et al., 2005, 2006). Recently, a genome-wide association study using data from the PRRS Host Genetics Consortium PRRS-CAP project discovered a quantitative trait locus (QTL) on chromosome 4 correlated with resistance, representing the latest progress on the genetics of PRRS resistance (Boddicker et al., 2012). Therefore, characterization of candidate genes and biological pathways associated with host PRRS resistance could benefit the prevention and control of PRRS (Huang and Meng, 2010; Kimman et al., 2009; Mateu and Diaz, 2008).

Zhang et al. (1999) identified a transcript encoding an ubiquitin-specific protease (UBP) that is specifically up-regulated upon viral infection. This was cloned and shown to be an ortholog of the murine ubiquitin-specific protease 18 (USP18) gene (Ait-Ali et al., 2009). More genetic and biochemical evidence suggest that protein ubiquitylation and deubiquitylation are of fundamental importance in the regulation of the innate and adaptive immune system (Liu et al., 2005; Sun, 2008). In humans, USP18 negatively regulates the JAK–STAT signaling pathway, and is therefore considered to be an inhibitor of type-I anti-viral signaling (Malakhov et al., 2002, 2003). In pigs, constitutive overexpression of *USP18* could restrict the replication and/or growth of PRRSV in part by altering the cellular distribution of two subunits of the NFκB heterodimers (p65 and p50) *in vitro*, highlighting the role of USP18 as a host restriction factor during innate immune response to PRRSV (Xu et al., 2012).

Our previous study used a Chinese indigenous pig breed, the Dapulian (DPL) pig. We demonstrated that after being infected with PRRSV, these pigs had a lower rectal temperature, a lower viral load, and less severe clinical symptoms than did Duroc × Landrace × Yorkshire (DLY) commercial pigs (Jiang et al., 2013). Using an Affymetrix porcine microarray, we also found that the mRNA expression of the *USP18* gene was significantly up-regulated in the lung tissue of infected DPL pigs but not in infected DLY commercial pigs (Xing et al., 2014), suggesting that *USP18* is probably the gene underlying PRRSV resistance in DPL pigs. In this study, we analyzed the *cis*-acting elements in the promoter region of the porcine *USP18* gene and found that the Chinese breed-specific allele A at site −1533 of the promoter region was responsible for the up-regulation of porcine *USP18* gene both prior to and post PRRSV infection.

2. Materials and methods

2.1. Animals, tissues, virus strain, and cell line

Sixteen DLY crossbred commercial pigs and 37 DPL pigs were randomly selected from the Bajie National Reserve Breeding Company of Pigs and the Conservation Center of Dapulian Pigs (Jining, Shandong, China), respectively. In addition, 112 Duroc pigs, 115 Landrace pigs, and 130 Yorkshire pigs were randomly sampled from large western pig populations that were reared under similar conditions in the Pig Breeding Center of Shandong Academy of Agricultural Sciences (Jinan, Shandong, China). A further 60 Laiwu pigs and 60 Lulai Black pigs were sampled randomly from the Laiwu Pig Preservation Center (Laiwu, Shandong, China). Genomic DNA was extracted from ear tissues using TIANamp Genomic DNA Kit (TIANGEN Biotech, Beijing, China) according to the manufacturer's instructions and stored at −20 °C. The concentration of genomic DNA was determined by electrophoresis and a UV spectrophotometer (Eppendorf, Hamburg, Germany).

A type 2 North American PRRS strain, JXA1, which was provided by the Laboratory of Immunobiology in Shandong Agricultural University was propagated in MARC-145 cells that were cultured and maintained in Dulbecco's Modified Eagle's medium (DMEM; Gibco, Carlsbad, USA). The titer of virus stock was 1×10^5 $TCID_{50}$/mL as determined by the cytopathic effect in MARC-145 cells.

2.2. Cloning and analysis of the promoter region

Based on the genomic sequence of the porcine *USP18* gene in the GenBank database (NCBI Reference Sequence: NC_010447.4), a pair of primers was designed to amplify a 2483-bp DNA fragment containing 22 bp of the 5′-untranslated region (5′-UTR) and 2461 bp of the promoter region of the porcine *USP18* gene from the DLY and DPL pigs. The primers were 5′-CGACGCGTCGGAGAAAGAAATCCAGTTGATGA-3′ (forward; the *Mlu*I site is underlined) and 5′-CCCAAGCTTGGGTATGCCTCCGTCCTCGCCT-3′ (reverse; the *Hin*dIII site is underlined). The PCR reaction was performed in a 20-μL volume containing 10 μL of 2× PrimeSTAR GC Buffer (Mg^{2+} Plus), 1.6 μL of dNTPs (2.5 mM each), 0.5 μL each of the primers (10 μM), 0.2 μL of PrimeSTAR HS DNA Polymerase (5 U/μL; TaKaRa, Dalian, China) and 1 μL of porcine genomic DNA, using the following cycling conditions: 94 °C for 5 min, then 32 cycles of 98 °C for 10 s, 61 °C for 15 s, and 72 °C for 3 min; followed by 72 °C for 7 min. PCR products were purified using a Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA). The PCR products were cloned into the pJET1.2 vector (Fermentas, Burlington, Canada) and sequenced. The putative promoter sequences from DLY and DPL pigs were aligned using software DNAMAN version 5.2.2 (Lynnon Biosoft, San Ramon, USA).

2.3. Vector construction

The putative promoter regions cloned from DLY and DPL pigs were inserted upstream of the firefly luciferase gene of the pGL3-basic vector (Promega, Madison, USA) to generate reporter plasmids, pGL3-USP18DPL and pGL3-USP18DLY. The 2483-bp PCR product from DPL pigs was digested with *Mlu*I and *Hin*dIII restriction enzymes (Fermentas), gel-purified (AXYGEN, Union, USA), and cloned into the same restriction enzyme sites of the