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Genomic characterization and pathogenicity of a strain of type 1 porcine reproductive and respiratory syndrome virus

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ABSTRACT

The emergence of type 1 porcine reproductive and respiratory syndrome virus (PRRSV) has been noticed recently in China. In the present study, the complete genomic characterization of a strain of type 1 PRRSV (designated GZ11-G1) was described and its pathogenicity for piglets was analyzed. The results showed that the complete genome of GZ11-G1 with a size of 15,094 nt, excluding the poly (A) tails, shared 80.2–96.3% identity with the representative strains of type 1 PRRSV, and in particular, it had highest homology (96.3%) with Amervac PRRS, a live vaccine virus of type 1 PRRSV and SHE, a rescued virus from an infectious clone of Amervac PRRS virus. Compared with the vaccine virus, the nonstructural and structural proteins of GZ11-G1 displayed extensive amino acid variations except for its ORF5a. GZ11-G1 was clustered with the strains of type 1 PRRSV including Cresa3267, Cresa3249, Cresa3256, Olot/91, 9625/2012, ESP-1991-Olot91 and Amervac PRRS vaccine virus by further phylogenetic analysis. Moreover, GZ11-G1 was shown to cause fever, higher viremia and lung and lymph node lesions in piglets. Our findings indicate that GZ11-G1 is genetically related to type 1 PRRSV strains within the cluster formed by Cresa3267, Cresa3249, Cresa3256, Olot/91, 9625/2012, ESP-1991-Olot91 and Amervac PRRS vaccine virus, and it is a pathogenic for piglets. This study aids in understanding the genetic variation and evolution of type 1 PRRSV.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the most prevalent viral disease that affects swine production worldwide. This disease is characterized by reproductive failure in sows and respiratory diseases in all ages of pigs (Albina, 1997; Rossow, 1998; Wensvoort, 1993). Since its first emergence in the US and Europe (Keffaber, 1989; Wensvoort et al., 1991), PRRS has caused tremendous economic loss to the global swine industry (Neumann et al., 2005; Pejsak et al., 1997). The causative agent of this disease, PRRS virus (PRRSV), is first identified in Europe in 1991 and independently in the US in 1992 (Wensvoort et al., 1991; Benfield et al., 1992). To date, PRRS remains endemic in many countries in the world despite available commercial vaccines against PRRSV.

PRRSV is an enveloped, single-stranded positive sense RNA virus. The viral genome is approximately 15 kb in length, with 5′-untranslated region (UTR) and 3′UTR (Conzelmann et al., 1993; Meulenberg et al., 1997). Due to the genetic and antigenic differences (Mardassi et al., 1994; Meng et al., 1995; Nelson et al., 1993), PRRSV are divided into two genotypes—European type 1 and North American type 2, which share only 55–70% identity at the nucleotide level (Murtaugh et al., 1995; Nelsen et al., 1999). Based on ORF5 nucleotide sequences, the type 1 PRRSV can further be differentiated into three subtypes, pan European subtype 1 and East European subtypes 2 and 3 (Shi et al., 2010; Stadejek et al., 2008), and the type 2 PRRSV can be classified into at least nine distinct genetic lineages (Shi et al., 2010).

The genome of PRRSV consists of at least 12 overlapping open reading frames (ORFs) including ORF1a, ORF1b, ORF2a, ORF2b, ORFs3-7 (Conzelmann et al., 1993; Snijder and Meulenberg, 1998; Wu et al., 2001), and the recently discovered ORF5a and a short transframe (TF) ORF overlaping the nsp2-coding region of ORF1a in the +1 frame (Fang et al., 2012; Johnson et al., 2011). ORF1a and ORF1b, occupying three-fourths of the genome, encode two polyproteins—pp1a and pp1ab, which are predicated to be cleaved into 16 viral nonstructural proteins (nsp) (Fang et al., 2012; Snijder

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et al., 1994; den Boon et al., 1995; van Dinten et al., 1996; Wassenaar et al., 1997). These nsps are not only involved in viral replication and genomic transcription (Bautista et al., 2002; Fang and Snijder, 2010), but also play important roles in the modulation of host innate immune responses (Beura et al., 2010; He et al., 2015; Li et al., 2010a; Shi et al., 2011; Yoo et al., 2010), and in the pathogenicity and virulence of PRRSV (Kwon et al., 2008; Li et al., 2014a; Wang et al., 2008). ORFs 2a, 2b, ORF3 to 7, and ORF5a encode viral structural proteins including GP2a, E, GP3, GP4, GP5, M, N and ORF5a (Johnson et al., 2011; Mardassi et al., 1995; Meng et al., 1994; Meulenberg et al., 1995; Music and Gagnon, 2010; van Nieuwstadt et al., 1991; Wu et al., 2001). In addition, the nsp2 has recently been recognized to be an integral membrane protein of PRRSV as a structural protein (Kappes et al., 2013, 2015).

Within both type 1 and type 2, PRRSV shares extensively genetic variations with diversity of strains (Forsberg et al., 2002; Frossard et al., 2013; Meng, 2000; Murtaugh et al., 1998). Different strains of PRRSV have been shown to present the differential pathogenicity for pigs (Brockmeier et al., 2012; Li et al., 2010b; Zhou et al., 2009a). Moreover, the continuous evolution of PRRSV results in the generation of novel or variant strains that cause severe clinical syndromes (Halbur and Bush, 1997; Han et al., 2006; Karniychuk et al., 2010; Tian et al., 2007; Wang et al., 2015). Thus, the persistent variation and evolution of PRRSV is an intractable issue for the effective control of PRRS.

Type 2 PRRSV strains are the major pathogens that cause clinical outbreaks of PRRS in China (Gao et al., 2004; Li et al., 2007; Tian et al., 2007; Zhou et al., 2008, 2009b, 2014, 2015a; Zhou and Yang, 2010). Although type 1 PRRSV has been recognized in recent years (Chen et al., 2011; Zhou et al., 2015b), information relevant to genomic molecular characterization and pathogenicity of type 1 PRRSV remains limited in China. In the present study, we characterized an isolate of type 1 PRRSV from the serum samples of a pig farm with clinically respiratory symptoms of PRRS, and analyzed the genomic genetic variation of the virus and its pathogenicity for piglets.

2. Material and methods

2.1. Cells and virus isolation

Porcine pulmonary alveolar macrophages (PAMs) were prepared as described previously (Zhang et al., 2009), and maintained (RPMI) 1640 medium (Fisher Scientific, Waltham, MA, USA) with 10% fetal bovine serum (FBS, Hyclone Laboratories Inc., South Logan, UT, USA) under a humid 5% CO2 atmosphere. MARC-145 cells were cultured in GIBCOTM Dulbecco's Modified Eagle Medium (DMEM) (Fisher Scientific) with 10% FBS (Zhou et al., 2009b). Serum samples were collected from an affected pig herd with respiratory manifestations in 2011 in Guizhou province of China where the pig herds had never been vaccinated with modified live vaccines against PRRS. PAMs and MARC-145 cells were inoculated with the samples. The cells were observed daily for CPE, and the presence of type 1 PRRSV was confirmed by RT-PCR with the primers Ef5-F and Er5-R (Table 1). The isolate was designated as GZ11-G1, and its third passage on PAMs was used for genome sequencing.

2.2. RT-PCR amplification and sequencing of genome

When 80% of the PAMs in culture exhibited CPE, the cells were collected and frozen at −80 °C. The cell cultures were subjected to the extraction of total RNA with TransZol (Transgen, Beijing, China). The total RNAs were dissolved in Rnase-free water and stored in −80 °C.

Table 1
Primers used for amplification and sequencing of gene fragments of GZ11-G1.

Primer[a]	Primer sequence (5′-3′)	Location[b]	Length(bp)
Ef5-F	CCCAACATACCCAGCAGCA	13369	
Er5-R	GCACGGATGACAAAACATACGA	13875	507
5′-Outer-R	CCTGTTGAGGCAAAGGCGAGTTAG	722	–
5′- Inner-R	GTCGTTGGAGGAAGTTGTGATTGCC	512	–
E1F	ATGATGTGTAGGGTATTCCCCC	1	
E1R	TGGAGGTACTTACCATGCACGC	1158	1158
E2F	TCACTGAGTCCCCTGAGAACGG	957	
E2R	TGGGACAAATCCAGTGGTTCG	2538	1582
E3F	GTTGTGGTTTGGCAGTCGG	2391	
E3R	AAAAGCTGGGAGGTGGGAG	3592	1202
E4F	CAAGAAGATGTCACCCCCTCC	3419	
E4R	GCAGCCACAAAAGTGTCCGA	4497	1079
E5F	GATCCCAGTCAGGCTATCAAATG	4319	
E5R	GCCGCAGTTAGTAAAACAGAAGG	5641	1323
E6F	CCTGCCTTAACACCGTGAATG	5319	
E6R	CTCAAAAAGAGGGGTTGGTGTC	6910	1592
E7F	ATGTGGGGACTGAAAGGAAAAC	6765	
E7R	ATTTACCATCAGACACGGGGG	7984	1220
E8F	CTCAAGACACCAAGTCCGCA	7910	
E8R	CAAACACAAATCAAGCCCACAG	9402	1493
E9F	GCATTGACCATGACCCTGAG	9191	
E9R	GGCTGAACACAAGGTTACAATC	10510	1320
E10F	AAGTCCCTAAATAAATCCCGAGC	10372	
E10R	TAGTCAGTGTAGTCCTTGCCGTC	11645	1274
E11F	TTGAAAACACTGAGGATTGGGC	11579	
E11R	GAGAAACAGGGCTTACAGGCGA	13024	1446
E12F	ATTACCACCACCAAATAGACGGG	12899	
E12R	CATTGCTCAGCCGAAGTCCTC	14085	1187
E13F	ACTGGGTTTTCTCACAACAAGCC	13729	
E13R	TAATTTCGGTCACATGGTTCCTG	15095	1367
3′-Outer-F	TAACCGAGCATACGCTGTGAG	14466	–
3′-Inner-F	GGTAAAAACCAGAGCCAGAAGA	14600	–

[a] F denotes forward PCR primer; R denotes reverse transcription or reverse PCR primer.
[b] Numbers under this column means the nucleotide position within the genome of GZ11-G1.

Based on the complete genomic sequences of type 1 PRRSV from GenBank database, the 13 primer pairs were designed for RT-PCR amplification and sequencing (Table 1). Thirteen overlapped fragments covering the whole genome were amplified by RT-PCR. 5′UTR Full RACE Kit and 3′UTR Full RACE Kit (TaKaRa, Dalian, China) were used to amplify the 5′ untranslated region (5′UTR) and 3′ untranslated region (3′UTR) of the isolate genome. Primers specific for the 5′UTR and 3′UTR of the isolate were listed in Table 1. Each PCR product was purified with the EasyPure Quick Gel Extraction Kit (Transgen) and cloned into *pEASY*-blunt cloning vector (Transgen) according to manufacturer's instructions, respectively, and then submitted to Invitrogen (Beijing, China) for sequencing.

2.3. Comparative sequence analysis

Genome analyses were conducted using the DNASTAR package and CLUSTAL X 2.1. The phylogenetic analyses were performed with the MEGA 4 program (Tamura et al., 2007). The phylogenetic trees were constructed from the nucleotide sequences with the Neighbor-Joining method, and the robustness of phylogenetic construction was evaluated by bootstrapping with 1000 replicates. Fifty-two strains of type 1 PRRSV available in GenBank were used for comparative sequence analyses in this study, including Lelystad virus (LV) (M96262) (Meulenberg et al., 1993), EuroPRRSV (AY366525) (Ropp et al., 2004), SD01-08 (DQ489311) (Fang et al., 2006); SHE (GQ461593) (Zhang et al., 2008); KNU-07 (FJ349261) (Nam et al., 2009); 01CB1 (DQ864705) (Amonsin et al., 2009); 07V063 (GU737264) (Delrue et al., 2010); Lena (JF802085) (van Doorsselaere et al., 2010); NMEU09-1 (GU047345) (Liu et al., 2010); Cresa3266 (JF276434), Cresa3267 (JF276435), Cresa3249 (JF276433), Cresa3256 (JF276432), Cresa3262 (JF276431) and