Virology 497 (2016) 111–124

Contents lists available at ScienceDirect

Virology

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

Construction and characterization of a full-length cDNA infectious clone of emerging porcine Senecavirus A

Zhenhai Chen [a,1], Fangfeng Yuan [a,b,1], Yanhua Li [a,1], Pengcheng Shang [a], Robin Schroeder [c], Kelly Lechtenberg [c], Jamie Henningson [a,b], Benjamin Hause [a,b], Jianfa Bai [a,b], Raymond R.R. Rowland [a], Alfonso Clavijo [a,b], Ying Fang [a,b,*]

[a] Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, United States
[b] Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, United States
[c] Midwest Veterinary Services, Inc., Oakland, NE 68045, United States

ARTICLE INFO

Article history:
Received 5 April 2016
Returned to author for revisions
30 June 2016
Accepted 4 July 2016

Keywords:
Senecavirus A
Picornavirus infection
Infectious clone
Reverse genetics
EGFP reporter virus
Swine
Vesicular lesion
Vesicular disease
SVA pathogenesis

ABSTRACT

A full-length cDNA infectious clone, pKS15-01-Clone, was constructed from an emerging Senecavirus A (SVA; strain KS15-01). To explore the potential use as a viral backbone for expressing marker genes, the enhanced green fluorescent protein (EGFP)-tagged reporter virus (vKS15-01-EGFP) was generated using reverse genetics. Compared to the parental virus, the pKS15-01-Clone derived virus (vKS15-01-Clone) replicated efficiently *in vitro* and *in vivo*, and induced similar levels of neutralizing antibody and cytokine responses in infected animals. In contrast, the vKS15-01-EGFP virus showed impaired growth ability and induced lower level of immune response in infected animals. Lesions on the dorsal snout and coronary bands were observed in all pigs infected by parental virus KS15-01, but not in pigs infected with vKS15-01-Clone or vKS15-01-EGFP viruses. These results demonstrated that the infectious clone and EGFP reporter virus could be used as important tools in further elucidating the SVA pathogenesis and development of control measures.

1. Introduction

Senecavirus A (SVA), formally named as Seneca Valley virus (SVV), is a single-stranded non-enveloped RNA virus. SVA belongs to the genus *Senecavirus*, family *Picornaviridae* (Adams et al., 2015; Hales et al., 2008). Important members in the family also include poliovirus, rhinovirus, hepatitis A virus, foot-and-mouth disease virus (FMDV) and swine vesicular disease virus (SVDV) (Graves, 1973; Inoue et al., 1989). The genome of SVA is a positive-sense RNA molecule ~7.3 kb in length. It contains a single open reading frame (ORF), encoding a large polyprotein, flanked by a long 5′ untranslated region (UTR; ~668 nucleotides), and a short 3′ UTR (~68 nucleotides) followed by a poly(A) tail. The viral polyprotein is predicted to be processed by virus-encoded proteases into 12 polypeptides in the standard picornavirus L-4-3-4 layout, with viral structural proteins encoded towards the 5′ end of the genome, while non-structural proteins encoded at the 3′ end (Hales et al., 2008; Rueckert and Wimmer, 1984). Primary cleavage events are predicted to involve a ribosome-skipping mechanism to separate P1-2A from 2BC-P3 (Donnelly et al., 2001) and a traditional proteolytic process by 3C protease to cleave between L and P1 and between 2BC and P3 (Hales et al., 2008). In comparison with other picornaviruses, sequence analysis of prototypic strain SVV-001 showed that the P1, 2C, 3C and 3D polypeptides regions were most closely related to those of cardioviruses, but other regions of the polyprotein differed considerably from those of the other known picornaviruses (Hales et al., 2008). Within its 5′ UTR, the SVA RNA genome contains an internal ribosome entry site (IRES), which displays the secondary structural features that resemble the IRES element (type IV IRES) of classical swine fever virus (CSFV) in the family *Flaviviridae*, suggesting recombination events might be occurred between the genomes of the *Picornaviridae* and *Flaviviridae* during persistent co-infection in pigs (Willcocks et al., 2011).

The first identification of SVA, known as SVV-001 isolate, was reported in 2002 from a PER.C6 cell culture. Thereafter, the virus

* Corresponding author at: Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, United States.
E-mail address: yfang@vet.k-state.edu (Y. Fang).
[1] Zhenhai Chen, Fangfeng Yuan, Yanhua Li contribute equally to this work.

http://dx.doi.org/10.1016/j.virol.2016.07.003
0042-6822/

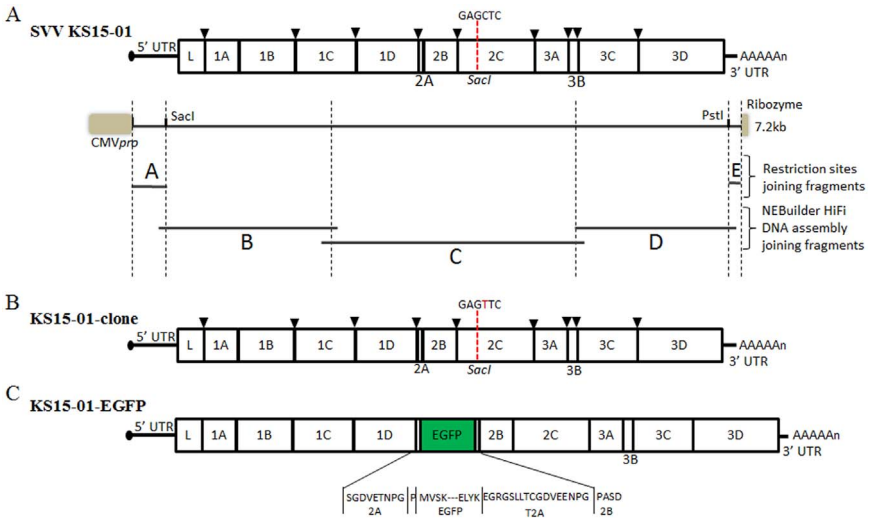

Fig. 1. Schematic diagram of the full-length SVA genome and construction of the full-length cDNA clones. (A) SVA genome organization and strategies of assembling the full-length cDNA clone. Top scheme: Genome organization of SVA. The ORFs are flanked by 5′ and 3′ UTR followed by the poly(A) tail at the 3′ end. Arrows indicate the polyprotein processing site by 3C protease. Dotted red line showing the SacI restriction enzyme site for introducing the mutation in cloned virus in panel B. Bottom scheme: Five separate genomic fragments were amplified and assembled into the pACYC177 vector using the unique restriction enzyme sites and the NEBuilder HiFi DNA Assembly Method (see details in Materials and Methods section). The full-length viral genome is under the control of a CMV promoter and followed by a HDV ribozyme. (B) Genome organization of the cloned virus vKS15-01-Clone with SacI site inactivated in 2C region. (C) A scheme of the reporter virus genome with an EGFP-T2A fusion gene inserted between 2A and 2B. T2A: teschovirus 2A peptide.

was developed as an oncolytic agent due to its selective tropism for human tumor cells and also no observed pathogenicity in human and animals (Hales et al., 2008; Reddy et al., 2007). Subsequently, sporadic serologically similar SVA isolates have been identified from pig samples in the US and Canada (Hales et al., 2008; Knowles and Hallenbeck, 2005; Pasma et al., 2008). Phylogenetic analysis suggested those different isolates of SVA had a common ancestor (Knowles et al., 2006). Historically, the association of SVA with swine vesicular disease was speculative, since the virus had also been isolated from pigs without clinical symptoms, and experimentally inoculating pigs with SVA isolates were unable to reproduce the disease (Hales et al., 2008; Knowles et al., 2006; Pasma et al., 2008; Yang et al., 2012). Recently, case reports from Brazil, Canada, China and the US provided evidence that SVA is a potential causative agent of idiopathic vesicular disease in pigs (Leme et al., 2015; Singh et al., 2012; Vannucci et al., 2015; Wu et al., 2016; Zhang et al., 2015). In some of those pigs tested as SVA positive, clinical signs of anorexia, lethargy, lameness, and vesicular lesions were observed. Gross lesions could be found on the oral mucosa, snout, nares, distal limbs, especially around the coronary bands (Singh et al., 2012). These clinical presentations resemble those caused by other economically more devastating transboundary pathogens that caused vesicular disease, including vesicular exanthema of swine virus (VESV), FMDV, and SVDV, which may lead to foreign animal disease investigations.

A reverse genetics system for SVA is needed to study the basic viral pathogenesis and develop modified live virus vaccines. Previously, a full-length cDNA infectious clone of SVV-001 was constructed (Poirier et al., 2012). However, it was developed as an anticancer agent, and the cloned viruses were not characterized for replication in pigs. In this study, we generated a full-length cDNA infectious clone of an emerging SVA, strain KS15-01. The *in vitro* and *in vivo* growth properties of the parental and cloned viruses were evaluated in cultured cells and nursery pigs. The availability of this infectious clone provides a powerful research tool for studying SVA pathogenic mechanisms and serves as a valuable backbone for future modified live virus vaccine development.

2. Materials and methods

2.1. Cells and viruses

PK-15 and BHK-21 cells were cultured in Minimum Essential Medium (MEM) (Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO), antibiotics [100 units/ml of penicillin (Gibco, Carlsbad, CA) and 100 μg/ml of streptomycin (Gibco, Carlsbad, CA)] and 0.25 μg/ml fungizone (Gibco, Carlsbad, CA) at 37 °C and 5% CO_2. The wild-type SVA was obtained from a diagnostic case submitted to KSVDL in 2015. Initially, the nasal swab samples were analyzed by next generation sequencing and SVA genome sequence was detected (Hause et al., 2016). Subsequently, the virus was isolated from a SVA positive nasal swab sample and plaque purified, designated as SVA KS15-01 (GenBank accession No. KX019804). The isolated virus was cultured on PK-15 cells. Recombinant viruses, vKS15-01-Clone and vKS15-01-EGFP, were rescued from transfected BHK-21 cells and passaged on PK-15 cells (see details below). For both wild-type and recombinant viruses, the passage two viruses on PK-15 cells were used for subsequent *in vitro* and *in vivo* experiments.

2.2. Construction of full-length cDNA clones of SVA KS15-01

In order to construct a full-length cDNA clone, the SVA KS15-01 was re-sequenced using Sanger sequencing method. A set of primers was designed based on the conserved genomic regions of a Canadian virus strain (GenBank accession No. KC667560; Table S1). These primers were used in RT-PCR to amplify viral RNA genome of KS15-01 virus. The PCR products were sequenced at Eurofin MWG Operon sequencing facility (Louisville, KY). The 5′- and 3′-end genomic sequences were further determined using GeneRacer® core Kit (Invitrogen, Carlsbad, CA) with specific primers (Table S1). To construct a full-length cDNA clone, five separate fragments, named A to E, flanked with unique restriction enzyme sites were amplified using Pfu Ultra High-fidelity DNA Polymerase (Agilent, Santa Clara, CA) and assembled together. As shown in Fig. 1, a cytomegalovirus (CMV) promoter was inserted upstream of the fragment A, while a hepatitis delta virus (HDV) ribozyme element was incorporated at the 3′-terminus of the viral