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PK-15 cells transfected with porcine CD163 by PiggyBac transposon system are susceptible to porcine reproductive and respiratory syndrome virus

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A B S T R A C T

The PiggyBac (PB) transposon system is a non-viral DNA-transfer system in which a transposase directs integration of a PB transposon into a TTAA site in the genome. Transgenic expression of porcine CD163 is necessary and sufficient to confer non-permissive cells susceptible to infection with porcine reproductive and respiratory syndrome virus (PRRSV). Such permissive cells can be used as a tool for PRRSV cellular receptor and other studies. One of the problems in studying PRRSV is the lack of porcine cell lines. In this study, efficient transfection and expression of porcine CD163 in PK-15 cells by PB transposition was demonstrated. The stable PK-15^{CD163} cell line was used in PRRSV infection assays. The data indicated that the average PB transgene copy number per genome was approximately 10. In line with previous literature the integration of PB into the genome had a bias toward the TTAA chromosomal site. The PK-15^{CD163} cell line was susceptible to infection by different PRRSV strains and the virus grew to similar titers compared to the Marc-145 cell line. This simplification of PK-15^{CD163} cell line production will provide a valuable tool to facilitate PRRSV cellular receptor studies and to accelerate existing vectors for PK-15 cell-based gene transfer and expression.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS), with its etiologic agent of PRRS virus (PRRSV), is a large threat to the health of swine worldwide and is one of the most economically important diseases in the swine industry (Neumann et al., 2005). Current measures to control PRRS depend mainly on live attenuated or inactivated vaccines. However, there have been specific drawbacks concerning either the safety or efficacy of vaccines, thus there is an urgent need for a new generation of PRRSV vaccines (Gale et al., 2010). A better understanding of how PRRSV enters its host cell is essential to the development of new vaccines for the control and prevention of PRRS.

Recent progress in studying the entry of PRRSV into host cells has led to the identification of a series of cellular receptors and entry mediators. The development of protocols involving transient or stable transfection of cDNA encoding cellular receptor(s) into PRRSV non-permissive cells provides important tools for the fundamental study of the functions of cellular receptors and PRRSV infection in general (Calvert et al., 2007; Delrue et al., 2010). However, studies of PRRSV cellular receptors are hampered by the generally large size of the receptor gene and expressing this as a transgene. PRRSV research is also hampered by a lack of biological relevant (e.g. pig) cell lines. Being able to turn pig cells that for whatever reasons are non-permissive for PRRSV infection, receptive for infection through efficient expression of a receptor would greatly help in the study of this virus.

Transposon systems have been used for non-viral gene transfer and have demonstrated promise for genetic studies in a wide variety of species (Clark et al., 2007; Sherman et al., 1998; Su et al., 2012; Wang et al., 2008; Yant et al., 2000). Transposons are mobile genetic elements that transpose between vectors and chromosomes by a "cut and paste" mechanism. During the transposition

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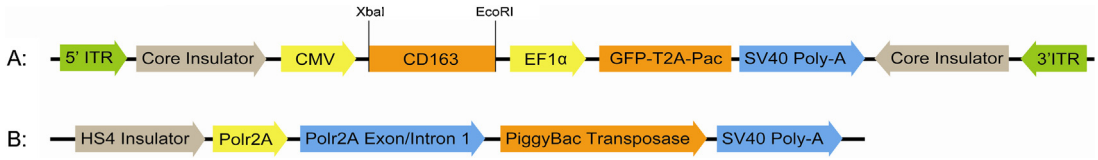

Fig. 1. Schematic diagram of the plasmids. (A) The donor plasmid: CD163-PiggyBac contains two expression cassettes (CMV-CD163 and EF1α-GFP-Pac) flanked by genomic core insulator and PB 5′-ITR sequences and 3′-ITR sequences. CMV, cytomegalovirus promoter; EF1α, human elongation factor-1 alpha promoter; T2A, insect virus Thosea asigna 2A sequences. (B) The helper vector: PB transposase expression plasmid. The PB transposase expression vector features a 5′HS4 insulator to support transcription from the rPolr2A promoter.

process, transposase recognizes transposon specific inverted terminal repeat (ITR) sequences located on both ends of the transposon vector and moves the contents from the original site and integrates them into the chromosomes. Owning to its natural property, a transposon vector containing a gene of interest between two ITR sequences can then transfer the gene from the vector to chromosome. The Sleeping Beauty (SB) is the first DNA transposon system that has been described to work in mammalian cells and is also the most widely used transposon system for mammalian transgenesis and mutagenesis (Takeda et al., 2007; Wang et al., 2008). For example, SB has been used to identify new cancer genes (Collier et al., 2005; Dupuy et al., 2006) and generate transgene animals (Kitada et al., 2009). However, SB transposition application is limited by its relatively low transposition efficiency and its nonspecific integration (Wu et al., 2006; Yant et al., 2005). Recently, PiggyBac (PB), a transposable element originally derived from the cabbage looper moth *Trichoplusia ni*, has been shown to work in mammals (Ding et al., 2005) and the activity of PB is higher than four tested transposon systems including two hyperactive versions of SB; Tol2 and Mos1 (Wilson et al., 2007; Wu et al., 2006). Furthermore, one important feature of PB transposon mediated gene transfer is that it can integrate more than 100 kb of foreign DNA into the chromosomes (Li et al., 2011). Given the high cargo capacity and high transposition efficiency in mammalian cells, PB has been used to introduce genes into and make transgenic cell lines (Lynch et al., 2010; Matasci et al., 2011; Xue et al., 2009), somatic transgene and mutagenesis (Landrette et al., 2011; Nakanishi et al., 2010), embryonic stem cells and induced pluripotent stem cells (Wang et al., 2008; Woltjen et al., 2009), gene therapy (Di Matteo et al., 2012; Nakazawa et al., 2011) and transgenic animals (Marh et al., 2012). However, PB transposition has not been well studied in pig cells or application for the study of virus infection.

Given the lack of biological relevant porcine cell lines to study PRRSV and knowledge of the receptor we decided to use the PB transposon system (Ding et al., 2005; Wilson et al., 2007) to generate a new resource to study this virus. Pig kidney (PK-15) cells have a wide range of applications in animal virus research, including gene delivery (Gao et al., 2007), virus infection (Calvert et al., 2007), and vaccine virus production (Delrue et al., 2010). CD163, a 130-kDa cell surface glycoprotein, functions as a cellular receptor for PRRSV. CD163 cDNA was introduced into PK-15 cells by plasmid transfection and was necessary and sufficient to render PK-15 cells susceptible to PRRSV (Calvert et al., 2007). In this study, we investigated and demonstrated that PB-mediated integration and expression of the gene encoding the cellular receptor CD163 cDNA in PK-15 cells rendered them permissive for PRRSV infection, thus providing a much needed resource to study virus biology.

2. Materials and methods

2.1. Construction of plasmid CD163-PiggyBac

The PB transposon system used in this study contains two vectors: a PB donor vector (System Biosciences, Mountain View, USA) as shown in Fig. 1A contains two expression cassettes: The first contains a multiple cloning site located downstream of the CMV promoter that allows for cloning porcine CD163 cDNA. The second, which is downstream, contains an EF1 alpha promoter driving the expression of green fluorescent protein (GFP) gene and puromycin-*N*-acetyl-transferase (Pac) gene to facilitate double selection in the mammalian cells. The two expression cassettes are flanked by genomic insulator elements for stabilized expression and PB ITR sequences for mobilization and integration. A helper vector (System Biosciences, Mountain View, USA) expressing PB transposase features a 5′ HS4 insulator to support transcription from the rPolr2A promoter (Fig. 1B).

Porcine CD163 cDNA (GenBank ID: JX292263) was cloned into the PB donor vector with the In-Fusion HD cloning kit (Clontech, Mountain View, USA) (Zhu et al., 2007): First, the donor vector was double-digested by XbaI (New England Biolabs, Ipswich, UK) and EcoRI (New England Biolabs, Ipswich, UK). Second, a pair of fusion primers for cloning porcine CD163 cDNA from porcine primary alveolar macrophage (PAM) cellular RNA was designed as described in the Clontech In-Fusion user manual with the following sequences: forward primer 5′-CATAGAAGAT**TCTAGA**ATGGTGCTACTTGAAGACTCTGG-3′ (overlap with donor vector underlined, XbaI site bold) and reverse primer 5′-ATTTAAATTC**GAATTC**TCATTGTACTTCAGAGTGGTCTCCT-3′ (overlap with donor vector underlined, EcoRI site bold). Finally, the CD163 fragment and the restriction enzyme-digested donor vector were mixed in one tube of In-Fusion HD Enzyme Premix and the reaction was incubated at 50 °C for 15 min. The reaction mixture was transformed into DH5α competent cells. Based on restriction digestion and sequencing, DH5α colonies containing plasmids with correct CD163 fragment were selected.

2.2. Cells culture, DNA transfection, viruses

Marc-145 cells obtained from the China Center for Type Culture Collection (CCTCC, Wuhan, China) and were propagated and maintained in Dulbecco's modified eagle medium (DMEM) (Life Technologies Corporation, Grand Island, USA) supplemented with 2–10% fetal bovine serum (FBS) (Life Technologies Corporation, Grand Island, USA) and 1% antibiotic-antimycotic (Life Technologies Corporation, Grand Island, USA). PK-15 cells purchased from CCTCC were grown in minimum essential medium (MEM) (Life Technologies Corporation, Grand Island, USA) supplemented with 10% FBS and 1% antibiotic-antimycotic. Lipofectamine 2000™ reagent (Life Technologies Corporation, Carlsbad, USA) was used as the transfection reagent. One day before transfection, 2×10^5 PK-15 cells were plated in 2 ml of growth medium per well in a six-well plate. PK-15 cells were co-transfected with 3 μg donor vector plasmid and different amounts (0.6 μg, 0.8 μg, 1.0 μg, and 1.2 μg) of helper vector plasmid. For the control transfection, PK-15 cells were only transfected with the donor vector plasmid. Viruses used in this study were the North American prototype PRRSV strain VR-2332 (GenBank ID: EF536003.1) (grown on Marc-145 cells), two highly pathogenic Chinese isolates of PRRSV SD16 (GenBank