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Inhibition of porcine circovirus type 2 replication by plasmid vector expressing siRNAs

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ABSTRACT: [OBJECTIVE] To find a means of controlling porcine circovirus 2 (PCV2) infection using siRNA. [METHOD] Three specific short interfering RNAs (siRNAs), two related to the replicase (rep) gene of porcine circovirus and one related to capsid (cap) gene of PCV2 were designed based on the genomic sequence of the virus. Corresponding DNA fragments were synthesized and annealed and ligated into the downstream of the mouse originated U6 promoter of the RNAi-Ready pSIREN-RetroQ ZsGreen vector. A gram-negative response
Recombinant plasmids were transformed into the host bacterium DH5a and positive clones were selected. The positive clones were sequenced and designated Retro-SH1, Retro-SH4, and Retro-SH6. These were transferred to Dulac cells both before and after infection with PCV2 and injected into BALB/c mice prior to and subsequent to PCV2 infection. [RESULTS] Transfer of 500 ng Retro-SH1, Retro-SH4 and Retro-SH6 prior to PCV2 infection was effective in inhibiting the replication of PCV2 in Dulac cells, with inhibition rates reaching 99% or better. Inhibition on the 10 strains of PCV2 isolated in clinical settings was similarly obvious, and there was little difference between strains. In vivo studies showed that a 10μg injection of the siRNA molecules described above had an inhibitory effect on the replication of PCV2 in mice, with inhibition rates between 26% and 99%. [CONCLUSION] Vector expressing siRNAs may become a new tool for preventing and controlling PCV2 infection.

Keywords: RNA interference; porcine circovirus 2 (PCV2); replication; inhibition

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Porcine circovirus 2 is a serious pathogen threatening pig producers around the world. PCV2 can cause various types of disease, including post-weaning multisystemic wasting syndrome, inflammation of the skin and nephritic syndrome[1], and infection by the virus can lead to compromised immune function causing secondary infections[2]. As such, the virus constitutes a major threat to swine producers. Presently, there are outbreaks occurring in multiple countries. There are also increasing reports of infections caused by a combination of PCV2 and another virus, and PCV2 infection and disease also exist among wild swine herds[3]. At present, there is no ideal means to inhibit the virus. There has been a long search for a new way to prevent or cure PCV2 infection, but because the proliferation of PCV2 in cells does not cause pathological changes to the cells, with immune compromise occurring after infection, research into a vaccine has as yet failed to achieve any fundamental breakthroughs. Consequently, there is an urgent need to explore new means of preventing and treating PCV2 infections.

PCV2 is a single stranded circular genome, containing 1767-1768 nucleotides along its length. It contains two major openreading frames (ORF), of which ORF1 codes two proteins related to the

replication of the virus, Rep and Rep′. These two proteins are necessary for the replication of the PCV2 virus, so that mutating the Rep and Rep' proteins can reduce the DNA replication of the virus while causing its protein synthesis to fall by 90% or more. The ORF2 gene codes the capsid protein for the virus. It is a major structural protein as well as an important immunogenic protein[4]. The genes coding these two proteins may act as target genes for silencing.

RNA interference (RNAi) is a phenomenon initiated by dual-chain RNA which selectively breaks down gene products with the same sequence after transcription. Formerly called post transcriptional gene silencing[15], RNAi is a new tool in research on genetic function, cancer treatments, and anti-virals[16]. To date, RNAi technique has been successfully applied to HIV, rmv, HCV and the flu virus, and represents a new means to prevent and treat viral infections.

The present study utilized the replication-associated protein (rep) and structural capsid protein (cap) as targets, designing and synthesizing siRNA and building a corresponding expression vector. The study assessed the inhibitory effect of various siRNA molecules on viral replication in cells and in experimental mice, in order to lay a foundation for the use of RNAi technique as an inhibitor for PCV2 infection.

1 Materials and Method

1.1 Materials

1.1.1 Strains, virus and vectors: RNAi expression vector: RNAI Ready pSIREN-RetroQ Zs Green Vector test kit, purchased from Bioscience Clontech, USA; E. coli strain: DH5α purchased from Invitrogen, USA; Porcine circovirus 2: viral isolate XSC strain (GenBank registration no. DQ104422) gift of Professor Puyan Chen, Nanjing Agricultural University, and 10 strains of PCV2 isolated by the researchers, identified and stored.

Identification of the PCV2 isolated strain XSC and the 10 additional PCV2 isolated strains were done as per methods reported in the literature[10]; Continuous Dulac cell lines without PCV1 infection, from the China Institute of Veterinary Drug Control; BALB/c mice, purchased from the Center for Comparative Medicine, Yangzhou University.

1.1.2 Major reagents: TaqMan Universal PCR Master Mix, purchased from Applied Biosystems, USA; Transfection agent: Sofast™, purchased from Xiamen Sunma Biotechnology Co., Ltd; Restriction enzymes: EcoR and BamHI, purchased from Takara Biotechnology (Dalian) Co., Ltd; other reagents were analysis-grade products purchased in China.

1.2 siRNA molecule design