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Data in Brief

Function analysis of proteins encoded by ORFs 1 to 8 of porcine circovirus-like virus P1 by microarray assay

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ABSTRACT

Porcine circovirus-like agent P1 is a newly discovered virus containing a single-strand circular genome. The genome of P1 is a DNA molecule of 648 nucleotides which contains eight open reading frames (ORFs) that probably encode potential proteins or polypeptides. Thus it is very important to clarify these proteins' function. Here we provide the methods and analysis of microarray data in detail to characterize the transcriptome profile of P1 with and without the ORF. The relevant microarray data sets have been deposited in Gene Expression Omnibus (GEO) database under accession number GSE71945.

Specifications	
Organism/cell line/tissue	Porcine kidney cells (PK15)
Sex	N/A
Sequencer or array type	Affymetris pig gene 1.1 ST Array
Data format	Raw
Experimental factors	PK15 cells transfected with the ORFs mutant plasmids and parental clone control
Experimental features	Identify gene expression profiles in the PK15 cells at 12-hour post-transfection
Consent	N/A
Sample source location	N/A

1. Direct link to deposited data

The deposited data can be found at: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE71945

2. Experimental design, materials and methods

Porcine circovirus (PCV), a member of the genus *Circovirus* in the family Circoviridae, is a small non-enveloped virus with a single-stranded circular DNA genome of approximately 1.8 kb [1]. To date, two distinct genotypes of PCV have been identified: PCV1 and PCV2. PCV2 has been regarded as a major pathogen of PCV associated diseases (PCVAD), of which postweaning multisystemic wasting syndrome (PMWS) is the major syndrome, having a severe economic impact on swine production wordwide [2,3].

PCV-like agent P1, discovered in 2007 from the serum of the porcine with PMWS, contains a circular DNA molecule of 648 nucleotides in length, which is highly homologous to the genome of PCV2 with the exception of 16 nucleotides [4]. Our previous study confirmed that the P1 virus has been epidemic in China's pig farms and can infect pigs displaying PMWS-like signs [5,6]. The genome of P1 contains eight open reading frames (ORFs) [7]. In this work, functions of the viral proteins of P1 were studied by microarray assay.

2.1. Construction of the molecular clone of P1

A tandem dimmer of P1 genome was cloned into the *Bam*HI site of Bluescript plasmid (Invitrogen), which was regarded as the "parent" to construct all other P1 mutant plasmids. The targeted RNA from a specific ORF would no longer be generated by specific mutation. Meanwhile, it did not cause any amino acid change in other overlapping ORFs.

2.2. Transfection, RNA isolation and microarray hybridization

PK15 cells seeded in 6-well tissue culture plates were transfected with the plasmids using lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. We have designed nine experimental groups, each containing 3 repeats. At 12 post-transfection, total RNA was extracted from the cells using TRIzol (Invitrogen) following the manufacturer's recommendations. The quantity and quality of each RNA sample were evaluated using a NanoDrop spectrophotometer (Thermo Scientific, USA).

The total RNA was used to synthesize double-strand cDNA and in vitro transcribed to cRNA, which was used to synthesize 2nd-cycle

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cDNA and then hydrolyzed by RNase H using Ambion WT Expression Kit for Affymetrix GeneChip (Affymetrix, Lot. No. 4011974, USA), and then 2nd-cycle cDNA was fragmented and the single-stranded cDNA was labeled with WT Terminal Labeling Kit (Affymetrix, Lot. No. 900671) and hybridized on Porcine Gene 1.1 ST Array Strip (Affymetrix, Lot. No. 901798). The hybridized arrays were washed and stained on the GeneAtlas Fluidics Station (Affymetrix). The fluorescent signals were detected with a GeneChip Scanner 3000 (Affymetrix).

2.3. Microarray data analysis

The scanned images were first assessed by visual inspection then analyzed to generate CEL files using the default setting of Affymetrix® GeneAtlas™ Workstation Software. Then the raw data (.CEL files) were normalized and summarized with the Affymetrix Microarray Suite 5.0 (MAS5) and with the Robust Multi-array Average (RMA) algorithm [8]. In a comparison analysis, a two class unpaired method in the Significant Analysis of Microarray software (SAM, version 3.02) was applied to identify significantly differentially expressed genes between TEST and CONTROL groups. Q value was set to <0.05 and transcripts were filtered on the basis of ≥1.2-fold difference.

The microarray CEL files, normalized data and experimental information have been deposited in NCBI's Gene Expression Omnibus database under accession number GSE71945.

2.4. Function enrichment analysis

Based on hypergeometric distribution, all differentially expressed genes were analyzed using two databases, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, and statistical significance was calculated when their q-values were <0.05.

2.5. Basic analysis

Here, we described that functions of the proteins encoded by ORFs 1 to 8 were characterized by microarray. Among these, by KEGG pathway analysis, the pathways involved in the digestive gland (gastric acid, salivary and pancreatic) secretion and the neurodegenerative diseases (Alzheimer's disease and Huntington's disease) induced by ORF1 were the most interesting.

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