RESEARCH ABSTRACT

SWINE HEALTH

Title: Enhancement of *in vitro* replication efficiency of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in MARC-145 cell line - **NPB #06-161**

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Scientific abstract

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most serious problems in the global porcine agricultural industry. Although several PRRS vaccines, including both modified Lelystad virus and killed virus, are currently available in the market, the efficacy of these vaccines is mainly based on the genetically diverged field strains of PRRSV and do not confer protection against a virulent heterologous strain. Specific vaccine against designated strains maybe a solution, but it is found that some strains of PRRSV isolated from field fail to propagate in cell culture, which make the vaccine production difficult. In this project, a PRRSV-susceptible cell-line derived from African green monkey kidney cell, MARC-145, was modified to enhance the susceptibility to various strains of PRRSV. Sialoadhesin (CD169) is a lectin-like receptor and is restrictedly expressed in subsets of tissue and inflammatory macrophages. It is shown that sialoadhesin mediates endocytosis and internalization of bound PRRSV particle into porcine alveolar macrophage. Transcripts of sialoadhesin were isolated from the porcine alveolar macrophage and a 5.4kb DNA fragment was obtained by RT-PCR. The fragment was cloned into expression vector to form a pcDNA3.1-sialo expression construct. Wild-type MARC-145 cells were then transfected with the construct and extrinsic sialoadhesin expression was detected with real-time RT-PCR assay. Both EU and NA PRRSV strains were inoculated into modified MARC-145, wild-type MARC-145 and MARC-145 transfected with vector only. ORF5 specific real-time PCR reveals that the expression of extrinsic sialoadhesin did not enhance the virus replication rate of both PRRSV strains. The reason may be due to that both these two virus strains used in the experiment have been already adapted to the wild-type MARC-145 cells, therefore these viruses are able to replicate efficiently and grow well in the MARC-145 cells. As these strains may use the intrinsic putative PRRSV receptor in the MARC-145 cells efficiently, the enhancing effect of the extrinsic sialoadhesin may be hampered and therefore there was no significant increase in the rate of replication. In order to further characterize the function of this extrinsic sialoadhesin, virus strains that replicate poorly in the wild-type MARC-145 should be isolated and screened for this experiment.

Nevertheless, this approach may take advantages from both PAM and MARC-145 cells and provide an alternative method of continuous *in vitro* PRRSV propagation.