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Research paper

Proteomic alteration of Marc-145 cells and PAMs after infection by porcine reproductive and respiratory syndrome virus

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ABSTRACT

Viral infections usually result in alterations in the host cell proteome, which determine the fate of infected cells and the progress of pathogenesis. To uncover cellular protein responses in porcine reproductive and respiratory syndrome virus (PRRSV), infected pulmonary alveolar macrophages (PAMs) and Marc-145 cells were subjected to proteomic analysis involving two-dimensional electrophoresis (2-DE) followed by MALDI-TOF-MS/MS identification. Altered expression of 44 protein spots in infected cells was identified in 2D gels, of which the 29 characterised by MALDI-TOF-MS/MS included 17 up-regulated and 12 down-regulated proteins. Some of these proteins were further confirmed at the mRNA level using real-time RT-PCR. Moreover, Western blot analysis confirmed the up-regulation of HSP27, vimentin and the down-regulation of galectin-1. Our study is the first attempt to analyze the cellular protein profile of PRRSV-infected Marc-145 cells using proteomics to provide valuable information about the effects of PRRSV-induced alterations on Marc-145 cell function. Further study of the affected proteins may facilitate our understanding of the mechanisms of PRRSV infection and pathogenesis.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically significant viral diseases of swine and a frustrating challenge to the global swine industry. It is characterised by severe reproductive failure in sows and respiratory distress in growing pigs and piglets (Wensvoort et al., 1992). Porcine reproductive and respiratory syndrome virus (PRRSV), the causative agent of PRRS, is a member of the Arteriviridae family. This family is composed of a group of positive (+) sense, single-stranded RNA viruses including simian hemorrhagic fever virus (SHFV), equine arteritis virus (EAV), and lactate dehydrogenase-elevating virus (LDV). PRRSV has a highly restricted cell tropism, both in vivo and in vitro (Kim et al., 1993). The virus infects the African green monkey kidney cell line MA-104 and its derivatives, Marc-145 and CL-2621, in vitro. PRRSV preferentially infects cells of the monocyte/macrophage lineage, especially porcine alveolar macrophages (PAMs), in the natural host (Duan et al., 1997). In both PAMs and monkey kidney-derived cell lines, the virus enters through a mechanism of receptor-mediated endocytosis (Nauwynck et al., 1999).

Little is known about the molecular mechanisms of PRRSV pathogenesis. Complex and mutual virus–host cell interactions occur when a virus invades the host. However, most of the cellular functions affected by PRRSV infection are still unidentified; hence, a comprehensive study of the interactions between PRRSV and PRRSV-infected host cells was necessary.

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Proteomic analysis of host cellular responses to virus infection may provide new insight into cellular mechanisms involved in viral pathogenesis. To date, proteomic approaches, e.g., coupling two-dimensional electrophoresis (2-DE) and mass spectrometry (MS) (Blackstock and Weir, 1999), have been widely used to study mechanisms of viral infection through the comparative analysis of cellular protein profiles (Alfonso et al., 2004; Zheng et al., 2008; Ringrose et al., 2008). This procedure of comparing protein expression patterns of normal and infected cells can provide exclusive information about the response of host cells to viral infection. Proteomic changes in infected host cells have been studied for many pathogenic mammalian viruses, including human immunodeficiency virus type-1 (HIV-1), severe acute respiratory syndrome (SARS)-associated coronavirus, rabies virus, Nipah virus and African swine fever virus. The purpose of this paper is to analyze the changes in cellular proteins of Marc-145 cells and PAMs exposed to PRRSV. Furthermore, we also discuss the altered functions of Marc-145 cells and PAMs, induced by PRRSV infection.

2. Materials and methods

2.1. Virus and cell culture

PRRSV JL/07/SW used for this study was isolated from an intensive pig farm with a typical PRRS outbreak in Jilin province of China in 2007. A stock of the virus was the fifth passage cell culture prepared in Marc-145 cells with a titer of $10^{6.19}$ $TCID_{50}$/mL. Marc-145 cells were grown in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (FBS; Invitrogen), 100 U/mL Penicillin G, 100 mg/mL Na streptomycin sulfate, and 2 mM L-glutamine. Pulmonary alveolar macrophages (PAMs) were prepared using lung lavage technique as previously described (Wensvoort et al., 1991) with minor modifications from three 6-week-old specific-pathogen-free (SPF) piglets (Beijing Center for SPF Swine Breeding and Management) that were free of PRRSV, porcine parvovirus, pseudorabies virus, swine influenza virus and *Mycoplasma hyopneumoniae* infections.

2.2. Virus inoculation

PAMs were incubated for 12 h at 37 °C in 5% CO_2 in RPMI-1640 medium, and the nonadherent cells were moved by gentle washing with RPMI-1640 medium before inoculation. Then, the cells (PAMs and Marc-145 cells) were inoculated with the virulent PRRSV strain JL/07/SW at an input multiplicity of about 2 $TCID_{50}$/cell. The uninfected cells served as mock-infected cells. Viral propagation was confirmed by daily observation of the cytopathic effect (CPE) and indirect immunofluorescence assay. After 12, 24, 36, 48, 60 and 72 h, the infection rate was monitored by indirect fluorescent-antibody (IFA) staining of cells, numbers of infected cells were quantitated by fluorescence microscopy.

2.3. Extraction of cellular proteins

The collected cells were lysed with lysis buffer (8 M urea, 2 M thiourea, 4% CHAPS, 40 mM Tris, 0.5% IPG buffer) containing complete protease inhibitor cocktail tablets (Roche). Approximate 5×10^7 cells were lysed in 1 mL lysis buffer. After vortex vigorously, the solution was centrifuged at 20 000 × *g* for 1 h at 4 °C. Uninfected cells were treated in parallel in the same way. The protein concentration was determined by the Coomassie Plus—The Better Bradford Assay Kit (Pierce Biotechnology, Rockford). The clear supernatants were collected and stored at −80 °C until use to prevent protein degradation. Three flasks of uninfected cells, as controls, were treated in parallel in the same way.

2.4. Two-dimensional gel electrophoresis

Approximately 300 μg proteins were loaded for first-dimensional separation. The samples were analyzed by 2-DE using commercial IPG strips (pH 4–7, 18 cm) (GE Healthcare) for IEF and standard vertical SDS-PAGE (12% acrylamide:bisacrylamide) for second dimension. IPG strips were rehydrated in a rehydration buffer consisting of 7 M urea, 2 M thiourea, 2% CHAPS, 65 mM DTT and 0.5% IPG buffer 4–7 for 13 h at room temperature with passive rehydration. Focusing was carried out at 20 °C with the current limited to 50 μA/strip using IPGPhor II (GE Healthcare). The program was performed as follows: 100 V for 1.5 h, 250 V for 0.5 h, 500 V for 1 h, 1000 V for 1 h, 3000 V for 1 h, 5000 V for 1 h, gradient ramping to 8000 V for 3 h, then 8000 V for a total of 60 000 Vh. Prior to the second dimension, IPG strips were equilibrated for 15 min with gentle shaking in equilibration solution I containing 6 M urea, 2% (w/v) sodium dodecyl sulfate (SDS), 30% (v/v) glycerol, 50 mM Tris–HCl (pH 8.8), reduced with 2% (w/v) DTT and a trace of bromophenol blue. After equilibration, proteins were separated on second-dimension separation, at a constant current of 1 w/gel for 30 min, then 10 w/gel until the dye reached the bottom of the gel. The strips were sealed on the top of the gels using a sealing solution (1% agarose, 0.5% SDS, 0.5 M Tris–HCl). The run was completed once the bromophenol blue reached the bottom or run of the gel. During the whole run, the temperature was set at 16 °C. Three independent cell cultures were conducted for biological replicates.

2.5. Gel staining and image analysis

The gels were stained by the modified silver staining method compatible with MS (Yan et al., 2000). The stained gels were scanned in an Image Scanner operated by LabScan 3.00 software. Image analysis was carried out with Image-Master 2D Platinum 6.0 according to manufacturer's protocol (GE Healthcare). At least three replicates from independent cultures were done for each point and data were analyzed by Student's *t*-test. Only the significantly differentially expressed protein spots ($p < 0.05$) with 1.5-fold different intensity or more were selected and subjected to identification by MS.