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RESEARCH ARTICLE

The cellular interactome for glycoprotein 5 of the Chinese highly pathogenic porcine reproductive and respiratory syndrome virus

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Abstract

The glycoprotein 5 (GP5) of porcine reproductive and respiratory syndrome virus (PRRSV) is a multi-functional protein that plays important roles in virus assembly, entry and viral anti-host responses. In the present study, we investigated the cellular binding partners of GP5 by using lentivirus transduction coupled with immunoprecipitation and mass spectrometry. There were about 40 cellular proteins identified with high Confidence Icons by MS/MS. Ingenuity Pathway Analysis (IPA) indicated that these proteins could be assigned to different functional classes and networks. Furthermore, we validated some of the interactions by co-immunoprecipitation (Co-IP) and confocal microscopy, including those with mitofilin, a mitochondrial inner membrane protein that might be involved in PRRSV or GP5-induced apoptosis, and calnexin, a protein chaperone that might facilitate the folding and maturation of GP5. The interactome data contribute to understand the role and molecular mechanisms of GP5 in PRRSV pathogenesis.

Keywords: PRRSV, glycoprotein 5 (GP5), interactome profile, mitofilin, calnexin

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiological pathogen of porcine reproductive and respiratory syndrome (PRRS) that was first recognized as “mystery swine disease” in the United States in 1987 (Keffaber 1989). Ever since, PRRS has become an economically devastating disease for swine agriculture all over the world. In particular, in 2006, emergence and extensive prevalence of the Chinese highly pathogenic PRRSV (HP-PRRSV) caused huge economic losses to the Chinese swine industry (Tian *et al.* 2007; Zhou and Yang 2010). This virus phylogenetically belongs to type II PRRSV, and displays enhanced virulence, characterized high morbidity and mortality as well as high fever (Tian *et al.* 2007; Zhou *et al.* 2009). Recent studies have revealed that the *in vivo* replication capacity, tissue distribution and immunomodulatory properties of PRRSV strains are related to the viral pathogenicity (Zhou *et al.* 2009; Li *et al.* 2012, 2014). However, most of the details await to be discovered.

PRRSV is a member of the genus *Arterivirus* in the family *Arteriviridae* (Cavanagh 1997) and has a genomic size of about 15 kb that contains at least ten overlapping open

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reading frames (ORFs) (Conzelmann *et al.* 1993; Johnson *et al.* 2011). Among its ORFs, ORF1a and ORF1b encode the viral replicase polyproteins pp1a and pp1a/b, which are further processed into at least 14 nonstructural proteins (nsps) including nsp1α, nsp1β, nsps2-6, nsp7α, nsp7β and nsps8-12 (Fang and Snijder 2010), and the others encode structural proteins (SPs) (GP2a, E, GP3, GP4, GP5, ORF5a, M and N) (Meulenberg *et al.* 1995; Johnson *et al.* 2011).

GP5 is an envelope glycoprotein with an apparent molecular mass of 25 kDa (Meulenberg *et al.* 1995; Dea *et al.* 2000) that plays important roles in both infectious viral particle assembly and PRRSV survival. In the viral envelope, GP5 forms a heterodimer with M that constitutes the major virion component (Meulenberg *et al.* 1995; Dea *et al.* 2000; Wissink *et al.* 2005). Consequently, deletion of either of ORF5 or ORF6 from an infectious PRRSV clone is lethal to the virus (Wissink *et al.* 2005). In addition, GP5 may play a critical role in the entrance of susceptible host cells (Delputte *et al.* 2005; Wissink *et al.* 2005). As well, GP5 was a major inducer of neutralizing antibodies *in vivo* (Wissink *et al.* 2003) and the N-linked glycosylations were able to weaken the immunogenicity of the nearby neutralization epitope (Ansari *et al.* 2006; Wei *et al.* 2012). Additionally, GP5 has been reported to be an apoptosis inducer and the first 119 amino acids constitute a region capable of fully inducing apoptosis (Suarez *et al.* 1996; Fernandez *et al.* 2002). Thus, PRRSV GP5 is a multifunctional protein.

In this report, we focused on the cellular binding partners of GP5 by using affinity purification (AP) combined with mass spectrometry (MS). The cellular proteins were identified and further confirmed by co-immunoprecipitation (Co-IP) and indirect immunofluorescence (IFA). These data would be helpful to further understand the molecular mechanisms of GP5 in PRRSV replication and viral pathogenesis.

2. Materials and methods

2.1. Cells and virus

Primary porcine pulmonary alveolar macrophages (PAMs) were prepared as previously described (Zhang *et al.* 2009) and maintained in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS) (Invitrogen). Human embryonic kidney (HEK) 293 cells and its derivative clone 293FT cells that were transformed with the SV40 large T antigen, and the African green monkey kidney epithelial cell line MARC-145 cells were cultured in DMEM (Invitrogen) containing 10% FBS. The highly pathogenic PRRSV strain, JXwn06, and its infectious cDNA clone plasmid (pWSK-JXwn) were used in this study (Zhou *et al.* 2009).

2.2. Antibodies and reagents

Mouse anti-HA monoclonal antibody (mAb) (H3663), rabbit anti-Myc polyclonal antibody (C3956) and MitoTracker Red (53271) were purchased from Sigma (Sigma, MO, USA). Mouse anti-GFP mAb (66002-1-Ig) and rabbit anti-mitofilin polyclonal antibody (10179-1-AP) were purchased from Proteintech (Chicago, IL, USA). Rabbit anti-calnexin polyclonal antibody (LS-B8413) was purchased from LifeSpan Biosciences, Seattle WA, USA. Mouse anti-GP5 and GP3 of PRRSV mAbs were prepared in our laboratory. GFP-Trap A Kit (gtak-20) with anti-GFP antibody conjugated to an agarose bead matrix for purification of the protein fused into GFP were purchased from ChromoTek (Planegg-Martinsried, Germany).

2.3. Plasmids construction

Coding region fragments of mitofilin and calnexin were amplified from the total RNA of PAMs by RT-PCR using the designed primers based on the sequences available in GenBank (XM_003481170.3, mitofilin; NM_001243210.1, calnexin), respectively. The amplified products were extracted using a RNAprep Pure Tissue Kit (TIANGEN, Beijing), according to the manufacturer's protocols. The reverse transcriptions were performed by using M-MLV reverse transcriptase (Promega, Madison, WI). The GP5 gene of PRRSV was amplified by PCR using pWSK-JXwn as templates. The plasmids including pCMV-Myc-mitofilin, pCMV-Myc-calnexin, pCMV-HA-GP5 and pEGFP-GP5 were constructed by conventional techniques. All the primers used in this study are listed in Table 1.

2.4. Preparation of GP5-expressing lentiviruses

The viral proteins-expressing lentiviruses were prepared as previously described (Dong *et al.* 2014; Wang X *et al.* 2014). Briefly, a lentiviral packaging system including pWPXL (12257), pMD2.G (12259) and psPAX2 (12260) were available from Addgene (Cambridge, MA, USA). The pWPXL-GP5-GFP and GFP-expressing plasmids were mixed with pMD2.G and psPAX2 with appropriate proportion, respectively, and co-transfected into HEK 293FT cells using the FuGENE HD transfection reagents (Roche Applied Science, Indianapolis, IN, USA). The cells were incubated at 37°C until the dishes were teemed with numbers of syncytias, and the supernatants containing lentiviruses were harvested and filtered with a 0.45 μm filter and concentrated by Amicon ultra-100 centrifuge tubes (Mick Millipore, Billerica, MA, USA). The titers of lentiviruses were determined using a QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV