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Research paper

Evolutionary characterization of pig interferon-inducible transmembrane gene family and member expression dynamics in tracheobronchial lymph nodes of pigs infected with swine respiratory disease viruses

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ABSTRACT

Studies have found that a cluster of duplicated gene loci encoding the interferon-inducible transmembrane proteins (*IFITMs*) family have antiviral activity against several viruses, including influenza A virus. The gene family has 5 and 7 members in humans and mice, respectively. Here, we confirm the current annotation of pig *IFITM1*, *IFITM2*, *IFITM3*, *IFITM5*, *IFITM1L1* and *IFITM1L4*, manually annotated *IFITM1L2*, *IFITM1L3*, *IFITM5L*, *IFITM3L1* and *IFITM3L2*, and provide expressed sequence tag (EST) and/or mRNA evidence, not contained with the NCBI Reference Sequence database (RefSeq), for the existence of *IFITM6*, *IFITM7* and a new *IFITM1*-like (*IFITM1LN*) gene in pigs. Phylogenic analyses showed seven porcine *IFITM* genes with highly conserved human/mouse orthologs known to have anti-viral activity. Digital Gene Expression Tag Profiling (DGETP) of swine tracheobronchial lymph nodes (TBLN) of pigs infected with swine influenza virus (SIV), porcine pseudorabies virus, porcine reproductive and respiratory syndrome virus or porcine circovirus type 2 over 14 days post-inoculation (dpi) showed that gene expression abundance differs dramatically among pig IFITM family members, ranging from 0 to over 3000 tags per million. In particular, SIV up-regulated *IFITM1* by 5.9 fold at 3 dpi. Bayesian framework further identified pig *IFITM1* and *IFITM3* as differentially expressed genes in the overall transcriptome analysis. In addition to being a component of protein complexes involved in homotypic adhesion, the *IFITM1* is also associated with pathways related to regulation of cell proliferation and *IFITM3* is involved in immune responses.

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1. Introduction

Domestic swine play an important role in human nutrition and economics since pork is the most consumed meat worldwide (http://www.fao.org/ag/againfo/themes/en/meat/backgr_sources.html). Moreover, pigs can harbor a number of zoonotic viruses of which influenza virus is the most important. Understanding how the pig responds to infectious disease may lead to better control of swine diseases that have a significant impact on pork production as well as human health. In addition, learning more about the pig immune response may lead to better animal models to study human disease. Viral respiratory diseases can cause dramatic losses in swine herds and are a major research focus worldwide. Recent advances in technology have enabled the efficient study of gene expression, which can be used to study the molecular pathogenesis and immunology of disease.

Innate antiviral immunity in the mammalian host is orchestrated by the interferon (IFN) system (type I, type II and type III) that plays a cardinal role in early detection and combat of invading viruses through IFN production and action. The interaction of virus and the host IFN-system potentially determines the outcome of most viral diseases (Gonzalez-Navajas et al., 2012; Katze et al., 2008). The interferon-induced transmembrane proteins (IFITMs) are a family of transmembrane proteins that respond differentially to IFN induction and viral infections. The IFITM genes are a subfamily in a larger family of transmembrane proteins called dispanins, which refers to a common two-transmembrane-helix protein structure (Sallman Almen et al., 2012); e.g., IFITM1 has been designated CD225. The current assembly of the human genome (Build 37.3) indicates there are five *IFITM* family members on chromosome 11: *IFITM1*, *IFITM2*, *IFITM3*, *IFITM5* and IFITM10. The mouse genome has six members: *IFITM1*, *IFITM2*, *IFITM3*, *IFITM5*, *IFITM6* and *IFITM10* located on chromosome 7, and a putative IFITM7 located on chromosome 16. While the multifunctional properties of IFITMs involved in embryo development, cell adhesion/growth and tumor progression are well described (Siegrist et al., 2011), the antiviral activities of IFITMs have only recently been studied. IFITM proteins can confer basal resistance to several viruses and are critical for the virustatic actions of IFN (Brass et al., 2009). Mouse and human IFITM3 expression has been shown to restrict influenza A virus (IAV) replication and IFITM1 and 2 appear to be important in hampering the replication of Marburg and Ebola filoviruses (Everitt et al., 2012; Huang et al., 2011). In addition, IFITM1–3 proteins were found to prevent infection of a growing list of viruses such as HIV-1, SARS, West Nile and Dengue fever (Brass et al., 2009; Everitt et al., 2012; Huang et al., 2011; Lu et al., 2011). Phylogenic analyses show species-specific diverse gene composition and potential functional divergence of vertebrate IFITMs (Hickford et al., 2012; Huang et al., 2011; Siegrist et al., 2011). It is unknown if the duplicated members have virus-specific recognition patterns and signaling pathways.

Our goal was to investigate the regulatory mechanisms and expression patterns of porcine *IFITMs*. In the study reported here, multiple *IFITM* genes were demonstrated to be differentially expressed in tracheobronchial lymph nodes (TBLN) during the course of infection with one of four common viral respiratory pathogens: porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), porcine circovirus type 2 (PCV2), and pseudorabies virus (PRV). Additional analyses demonstrated how many putative porcine IFTM family members exist and which are highly conserved human/mouse orthologs that may exert anti-viral activity.

2. Materials and methods

2.1. Manual annotation and bioinformatic analyses of porcine IFITM family

Porcine IFITM entries were extracted from the NCBI gene database (http://www.ncbi.nlm.nih.gov/gene/) and further curated using BLASTP against the current swine genome assembly (Sscrofa10.2) (Groenen et al., 2012). The domain structures of IFITM proteins were defined based on human IFITM entries in the Conserved Domain Database (Marchler-Bauer et al., 2013). The sequence alignment and conserved residues were analyzed with Jalview (Waterhouse et al., 2009), and the phylogenic analysis was performed with Mega5 (Tamura et al., 2011). The subcellular location of eukaryotic proteins was predicted using a hybrid approach (Hslpred, http://www.imtech.res.in/raghava/hslpred/) and the algorithms were based on single/multiple sites (EukmPloc (Chou and Shen, 2010)) or a decision tree of several support vector machines (MemLoci (Pierleoni et al., 2011)).

2.2. Virus, animals and experimental design

TBLN were collected from pigs that were part of 2 studies of virtually identical design conducted at the National Animal Disease Center (NADC), USDA, ARS, Ames, Iowa. Each study was designed to investigate the comparative global TBLN transcriptome profile of pigs infected with either PRV (Study 1), or SIV, PRRSV, or PCV2 (Study 2). The experimental design was similar for both studies and TBLN tissue was selected for study because the lymph from the lungs passes through these lymph nodes making them an active site in the immune response against pulmonary disease.

Prior to virus challenge at 4–5 weeks of age, pigs were determined to be free of PRV, SIV, PCV2, and PRRSV. On 0 days-post-inoculation (dpi) pigs received an intranasal challenge with 2 ml of either challenge virus or sham inoculum (control) prepared from the respective cell culture used to propagate challenge viruses. Each group consisted of 20 pigs and was housed in an BSL-2 isolation room from 0 to 14 dpi, the duration of the experiment. Five pigs from each group both infected and uninfected were euthanized and necropsied on 1, 3, 6 and 14 dpi and TBLN from each pig was collected immediately, minced and stored in RNAlater (Life Technologies Corporation, Grand Island, NY) at −80 °C until homogenized for RNA extraction.