Identification of host proteins that interact with non-structural proteins-1α and -1β of PRRSV-1

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Porcine Reproductive and Respiratory Syndrome Virus

The porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of arguably the most important infectious disease affecting the global pig industry.

PRRSV causes respiratory disease in piglets and reproductive failure in sows and consequently impacts both growing and breeding sectors.

PRRSV is endemic in most pig producing countries where it is responsible for major economic losses.

Both live attenuated and inactivated PRRSV vaccines are available and are widely used, however, they are falling to control PRRS panzootically.

Given the economic importance of PRRS, safer and more efficacious vaccines are urgently needed.

An improved understanding of virus-host interactions that lead to immunomodulation could aid the design of improved vaccines.

PRRSV non-structural proteins (NSPs)

PRRSV encodes at least 16 non-structural proteins (NSPs) which are involved in viral replication and/or modulating the host immune responses.

An improved understanding of virus-host interactions that lead to immunomodulation could aid the design of improved vaccines.

This project will therefore focus on improving our understanding of the modulation of host cell responses by NSP1α and NSP1β from PRRSV-1 subtype 1 strain 215-06.

NSP1α (shown in Figure 1) is required for subgenomic mRNA synthesis and has previously been shown to suppress type I IFN, TNFα and inhibit NF-κB signalling1.

Methods

- PRRSV-1 NSP1α and NSP1β were screened using the yeast-2-hybrid (y-2-h) system and a cDNA library generated from porcine alveolar macrophages (PAM) – the primary target cell for infection.
- The y-2-h system is a method of detecting protein-protein interactions. This is summarised in Figure 2.

Results

- Sequence analysis revealed 62 and 127 potential binding partners for NSP1α and NSP1β, respectively, which vary in function.
- 13 proteins from the NSP1α screen and 36 from the NSP1β screen were selected to take forward and confirm.
- Selected proteins are involved in either IFN signalling, the NF-κB pathway, ubiquitination or nuclear translocation.
- Putative interactions of selected proteins with NSP1β were retested using y-2-h with additional controls (Figure 4).
- 3 interactions from the NSP1α screen and 27 from the NSP1β screen were found to be genuine.

Future work

- Further confirm interactions using co-immunoprecipitation experiments and mass spectrometry.
- Use confocal microscopy to confirm interactions by looking for co-localization.
- Deletion/mutation analysis to identify exact amino acids/regions involved in interactions.
- Investigate if same interactions occur in a PRRSV-1 strain of higher pathogenicity, e.g. subtype 3 SU1-Bel strain.
- Characterize confirmed interactions using a range of biochemical techniques e.g. quantitative RT-PCR, luciferase reporter assays and Western blot analysis.

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References


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Figure 1: PRRSV NSP1α crystal structure6

Figure 2: An overview of the y-2-h system used to detect protein-protein interactions.

- Positive colonies, those that grew on media deficient in -T+LAM-Adh-His-Xa-Gal-His, were then selected and subject to the workflow in Figure 3.

Figure 3: An overview of how prey proteins were identified.

Grow up positive yeast colonies in -Leu broth

- Extract prey plasmids from yeast

PCR to amplify cDNA insert within in each prey plasmid

Run agarose gels to check presence and number of inserts

Purify PCR products that produce only 1 band

Prepare samples for sequencing

Sequence

Analyse sequences: translate using ExPaSy translate tool software and identify prey protein using NCBI protein BLAST software

Figure 4: Confirmation of NSP1α interactions in the y-2-h system. Test plate layout (A) and examples of cathespin B precursor as a genuine interaction (B) and anaphase-promoting complex subunit 10 as a false positive (C). α = selected protein; v = empty vector pgBKT7-T; T = pgAD77-T; S3 = pgBKT7-S3; LAM = pgBKT7-LAM. In the y-2-h system, T-Gs and T-LAM act as positive and negative controls, respectively.

Figure 5: Future work

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