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Virus Host Interactions in SARS Coronavirus Infection

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ABSTRACT

The global outbreak of Severe Acute Respiratory Syndrome which began in 2002 and ended in 2003 resulted in over 8000 cases in 29 countries, causing an atypical pneumonia which resulted in a case fatality rate of ~10%. The etiological agent of this disease was found to be a novel coronavirus dubbed SARS Coronavirus (SARS-CoV). One of the distinguishing features of this virus is the presence of several accessory genes in its positive-stranded RNA genome which have no known homologs in the coronavirus family. In this thesis we examine virus host interactions in the context of SARS coronavirus in order that some insight may be gained into the biology of this virus, which is still the subject of investigation.

A lot of work has been done to study the function of SARS-CoV accessory proteins. One of these is the ORF6 accessory protein, which has been shown to accelerate infection, interfere with interferon production and signaling by impeding nuclear translocation, induce membrane rearrangements in the host cell and induce ER stress and subsequent caspase-mediated apoptosis. Examination of the ability of ORF6 to interact with other virus proteins by a yeast-two-hybrid assay showed an interaction with the nsp8 replicase protein. A direct interaction was shown using a cell-free system, and subsequently, transient transfection and immunoprecipitation experiments in Vero E6 cells showed that these proteins interacted in mammalian cells, which was confirmed in SARS-CoV infected cells. Examination of the co-localisation of these proteins in infected Vero E6 cells also showed a subcellular localization of the ORF6 protein to a intracellular vesicular population which was Lamp1- and CD63-positive.

The impedance of nuclear translocation by the ORF6 protein is linked to its ability to function as an interferon antagonist. A plasmid-based system comprising a CMV-derived promoter was used to replicate this impedance, and it was found that the impedance is dose-dependent. Additionally, plasmid-expressed ORF6 was shown by immunofluorescence to colocalise with an intracellular vesicular population positive for Lamp1 and CD63. Alanine substitution of a putative diacidic motif in the ORF6 protein showed a reduction in the impedance of nuclear translocation and when examined by immunofluorescence also showed clustering of ORF6-stained vesicles, suggesting that the impedance of nuclear import by ORF6 and its localisation to this specific vesicular population are linked.

In order to characterize the differential expression of genes in host cells which might lead to ORF6-mediated apoptosis, a defective recombinant vaccinia virus expressing ORF6 was generated. Analyses were performed using a QPCR array specific to human apoptotic signaling as well as microarray analysis for examination of genome-wide differential expression. Subsequent analysis of these results suggested that the extrinsic pathway of apoptosis was upregulated in cells expressing ORF6, and that intracellular calcium flux might have a role to play in ORF6-mediated apoptosis.

The introduction of an exogenous nitric oxide donor into Vero E6 cells infected with SARS-CoV has been shown to exert a negative effect on viral replication. Here an attempt was made to explore the mechanism of this inhibition. Nitric oxide can exert an effect on viruses either directly or through several redox intermediates, one of which is peroxynitrite, which is formed through the reaction of nitric oxide and superoxide. It was shown that the effect of the exogenous nitric oxide donor SNAP on SARS-CoV replication was not due to the effect of peroxynitrite, but probably through direct action of nitric oxide or another derivative. Examination of the palmitoylation of the S protein in SNAP-treated cells showed that SNAP treatment was able to reduce S palmitoylation and consequently its binding to the ACE-2 receptor in an *in vitro* cell-cell fusion assay. Also, QPCR analysis of viral RNA production showed a significant reduction in SNAP-treated cells. Examination of the cleavage of viral replicase polyproteins in these cells showed an alteration in the cleavage pattern of the replicase polyproteins.

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