STK11IP identification in double membrane vesicle composition of HCoV-OC43 infected cells by subcellular fractionation

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Introduction

- According to the World Health Organization, over 4.9 million people have died globally from the SARS-CoV-2.1
- There is only one FDA approved therapeutic treatment called Remdesivir that functions as nucleoside analog RNA polymerase inhibitor.2
- There is a significant lack of understanding of the virus’s ability to exploit host cell mechanisms for viral replication and survival.
- Sharing the same genus, HCoV-OC43 can be used as a model viral strain for SARS-CoV-2.
- Following the osteopathic tenet of the body as a unit, this research investigates the dependence of HCoV-OC43 on host cell mechanisms for viral survival.

Methodology

- MA104 cells (African Green monkey kidney) were infected with human HCoV-OC43 or Mock infected in duplicate.
- Subcellular fractions of the nucleus, mitochondria, plasma membrane, and cytosol from cell samples were obtained using centrifugation (Figure 1).
- Samples were standardized by Coomassie stained gel electrophoresis.
- SDS-Page and transfers of the fractions where then performed and later used for viral and cellular protein probing with targeted antibodies.
- Antibody cellular markers were selected to confirm fractions from the Abcam database.
- Infection was established using COVID Spike in whole cell lysate and confirmed by cell culture cytoplastic effect (CPE).

Data Analysis

- Western blot chemi image analysis was used for cellular and viral protein band expression.
- Known antibodies for target proteins were used to confirm the identity of subcellular fractions in MA104 infected and uninfected cells.
- Patterns of interest were scored based on whether proteins appeared at their expected molecular weight in expected or unexpected fractions.
- Antibodies for mTOR were found in the uninfected nuclear fractions and infected mitochondrial fractions, while STK11 was not found in infected fractions.

Results

- Plasma membrane markers were found in infected cellular fractions of not only the predicted membrane fraction but also nuclear and mitochondrial fractions (Table 1).
- The same pattern seen in plasma membrane was seen with STK11IP infected fractions.
- Markers for mTOR were found in the uninfected nuclear fractions and infected mitochondrial fractions, while STK11 was not found in infected fractions.

Conclusion

- SARS-CoV replicates within the cytoplasm of infected cells through poorly understood double-membrane vesicles (DMVs) that form on host endoplasmic reticulum.
- Results support the theory that DMVs could be made of a mixture of modified host cell membranes including plasma and mitochondrial membranes as a defense mechanism for successful viral RNA synthesis.2,3
- Based on our previous research, increased expression of STK11IP, an AMPK pathway inhibitor, in DMVs suggest that STK11IP may be interacting with host plasma membranes for replication within DMVs.

References


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