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.

Figures & Tables

ANTIBODIES	Subcellular fractionation of HcoV-OC43 infected cells to identify STK11IP and viral protein targets within fractions							
	Nuclear INF	Nuclear MOCK	Mitochondria INF	Mitochondria MOCK	Membrane INF	Membrane MOCK	Cytoplasm INF	Cytoplasm MOCK
Ab76020 (Plasma Membrane)	+	+	+	-	+	-	-	-
Ab 52866 (Cytoplasm)	+	+	+	+	+	+	-	-
Ab 109110 (Early Endosome Marker)	+	+	+	-	-	-	-	-
STK11IP	-	+	+	-	+	-	-	-
mTOR	-	+	+	-	-	-	-	-
STK11	-	-	-	-	-	-	-	+
OC43- Spike	-	-	+	-	-	-	-	-

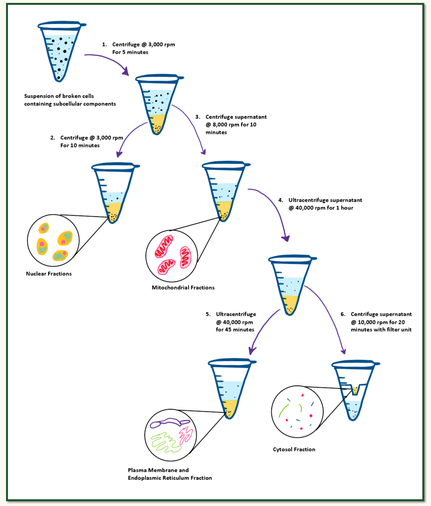

Figure 1: Subcellular Fractionation Protocol

Table 1. **Subcellular fractionation of HcoV-OC43 infected cells to identify STK11IP and viral protein targets within fractions.** Selected antibodies were used to target known host and viral proteins. Plasma membrane markers (Ab 76020) were found in infected cellular plasma membrane, nuclear, and mitochondrial fractions. Plasma membrane markers (Ab 76020) were found in uninfected (mock) samples of nuclear fractions only. Cytoplasm markers (Ab 52866) were found in both infected and uninfected nuclear, mitochondrial and plasma fractions but not in the infected or uninfected fractions predicted for cytoplasmic fractions. Early endoplasmic markers (Ab 109110) were found in infected nuclear and mitochondrial fractions and the uninfected nuclear fraction. STK11IP was identified within the uninfected cell sample for predicted nuclear fractions and infected mitochondrial and membrane fractions. Markers for mTOR were found in the uninfected nuclear fractions and infected mitochondrial fractions. STK11 was only found in the uninfected cytoplasmic fraction. OC-43 Spike protein was detected in Mitochondrial fractions of infected cells.

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-membranes 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. [3, 4]
- Based on our previous research, increased expression of STK11IP, an upstream regulator of STK11, modulates mTOR promoting viral replication by inhibition of the AMPK pathway.
- Our results show mirrored location markers for both plasma membrane and STK11IP, suggesting that STK11IP may be interacting with host plasma membranes for replication within DMVs.

References

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