Application Brief

Cell Analysis

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Investigating Effects of Respiratory Syncytial Virus Infection on Host Cell Mitochondrial Function

Introduction

Pathogenic viruses have different strategies to ensure ideal environments for their replication and spread, and have evolved to alter numerous host cell pathways. Recent studies have shown that many viruses induce large-scale alterations in host cellular metabolism, including hijacking of central carbon metabolic pathways and changes in mitochondrial function. Greater understanding of these metabolic alterations required for viral replication may lead to innovative therapeutic tactics through targeted modification of specific mitochondrial functions or pathways.

Respiratory syncytial virus (RSV) is a leading cause of respiratory tract illness in infants and is a threatening respiratory pathogen in immunosuppressed and elderly adults, leading to a greater number of deaths each year worldwide than influenza. Currently, there are no effective anti-RSV therapeutics generally available, highlighting the need for a deeper understanding of host-pathogen interaction.

Pneumoviruses, such as RSV, replicate in the cytoplasm. Consequently, effects of viral infection on host cell metabolism and bioenergetics have become a focus of recent research. This is driven by the fact that changes in cellular cytoskeletal organization and/or motor activities can impact mitochondrial distribution and function, as mitochondria are relocated intracellularly through the action of molecular motors (e.g., the dynein ATPase) operating on microtubules and actin filaments.

Agilent Seahorse XF technology delivers a real-time view of the fundamental processes of energy metabolism in live cells. This capability enables convenient interrogation of the metabolic implications of pathogen infection on host cells, including those specific to mitochondrial function, offering new insights on key host-pathogen interactions. These insights may, in turn, inform the development of novel therapeutic strategies.

Employing the utility of the Agilent Seahorse XF Cell Mito Stress Test to measure parameters of mitochondrial function, in combination with orthogonal assay methods of small interference RNAs (siRNAs), redox/membrane potential measurements, and high-resolution quantitative imaging/flow cytometric analysis, Hu *et al.*[1] have shown that that RSV drives a staged redistribution of mitochondria in infected cells. This redistribution is observed to occur in a microtubule and dynein-dependent fashion, concurrent with decreased mitochondrial respiration and increased glycolytic activity (Figure 1). These changes correlated with both time postinfection (p.i.) and initial dose (i.e., multiplicity of infection, m.p.), shown in Figures 1 and 2, respectively. Inhibiting this mitochondrial redistribution provoked by RSV infection with the microtubule-depolymerizing agent nocodazole (NCZ), or the dynein ATPase-inhibitor EHNA, resulted in a restoration of mitochondrial activity and protected against RSV infection. In conclusion, the authors' thorough study uncovers several novel influences of RSV infection on host cell mitochondria that are relevant to future therapeutic approaches. Specifically, the study shows that inhibitory agents of dynein/microtubule-dependent mitochondrial redistribution and/or mitochondrial reactive oxygen species (ROS) production limit RSV infection (Figure 3), with MitoQ in particular able to decrease viremia and airway inflammation in mice. This investigation shows that therapeutic modulation of host cell mitochondrial function represents a potential target to counteract RSV infection.

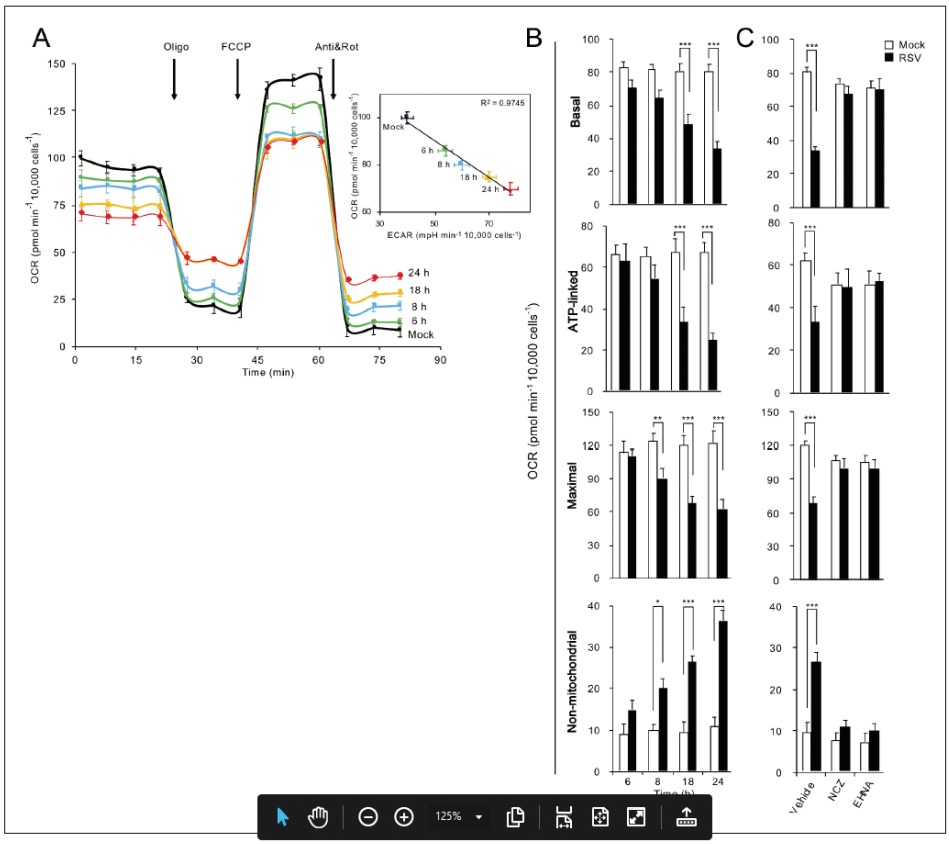

Figure 1. RSV infection inhibits host mitochondrial respiration in dynein/microtubule-dependent fashion. Cellular bioenergetic analysis was performed using the Agilent Seahorse XFe96 Extracellular Flux Analyzer. A549 cells were (A and B) mock-infected for 24 hours or RSV-infected (MOI 1) for 6 to 24 hours or (C) RSV-infected (MOI 1) for 18 hours with additions of the microtubule-depolymerizing agent nocodazole (NCZ, 17 μM), or the dynein ATPase-inhibitor EHNA (200 μM) over the last 2 hours. (A) An example of a typical oxygen consumption rate (OCR) obtained in these experiments. OCR was measured in real time upon sequential additions of ATP synthase inhibitor oligomycin (Oligo, 1 μM), proton ionophore FCCP (1 μM), mitochondrial complex III inhibitor antimycin A (Anti, 1 μM), and mitochondrial complex I inhibitor rotenone (Rot, 1 μM). Inset: Correlation of OCR, a measure of mitochondrial respiration, and extracellular acidification rate (ECAR), an indicator of glycolysis (R^2 = 0.9745). (B and C) Mitochondrial respiration function parameters of basal, ATP-linked, maximal, and nonmitochondrial respiration were determined. Results represent the mean ±SEM for n = 3 independent experiments, each performed in triplicate. ***p <0.001, **p <0.01, *p <0.05 compared to the mock-infected cells.

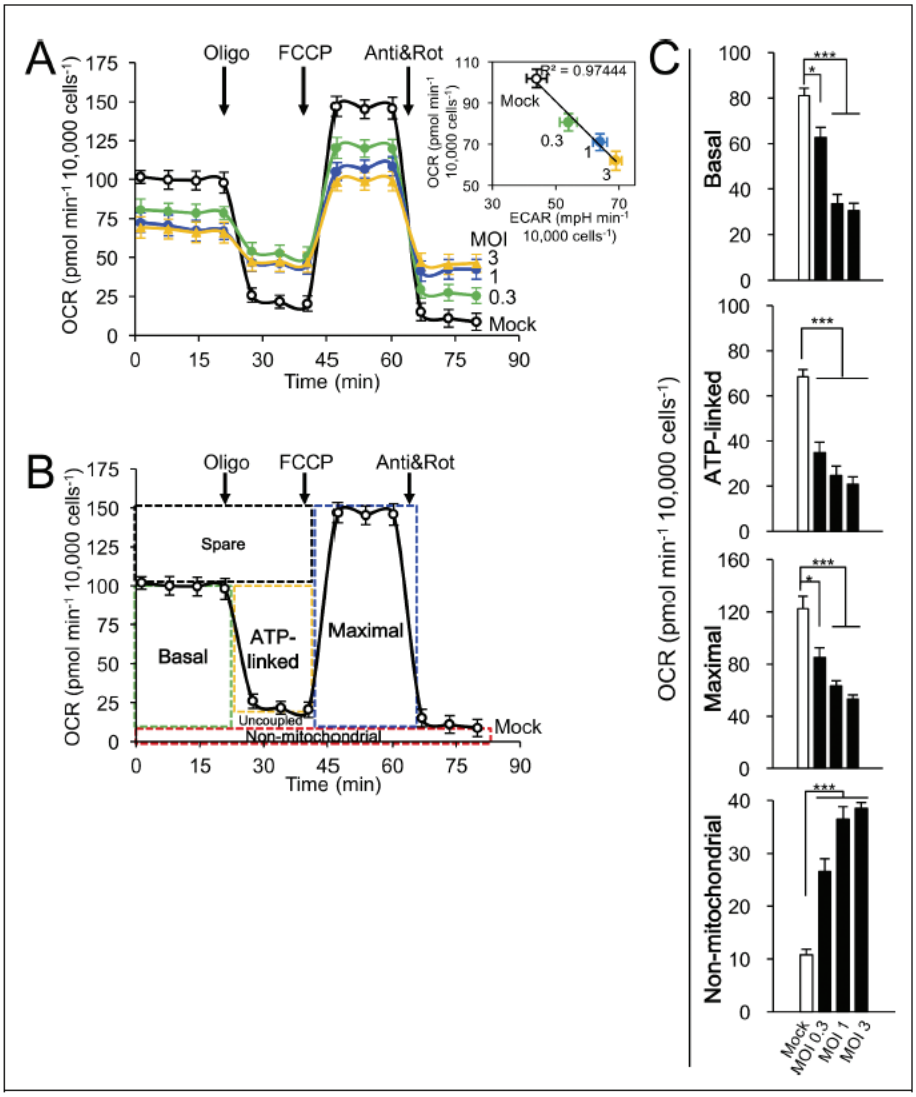

Figure 2. Correlation of multiplicity of infection (MOI) and effects on host cell mitochondrial respiration. Cellular bioenergetic analysis was performed using an Agilent Seahorse XF96 Extracellular Flux Analyzer. (A to C) A549 cells were mock- or RSV-infected (MOI 0.3 to 3) for 24 hours. (A) Measurements/analysis were performed as per Figure 1. Inset: Correlation of OCR and ECAR by linear regression (R^2 = 0.97444). (B) Schematic representation depicting the calculation of the basal, ATP-linked, maximal, and nonmitochondrial respiration parameters. (C) Pooled results were calculated as per Figure 1. ***p <0.001, *p <0.05 compared to the mock-infected cells.

References

1. Hu, M. *et al.* Respiratory Syncytial Virus Co-Opts Host Mitochondrial Function to Favor Infectious Virus Production. *Elife* **2019**, *8*, e42448. https://pubmed.ncbi.nlm.nih.gov/31246170/.

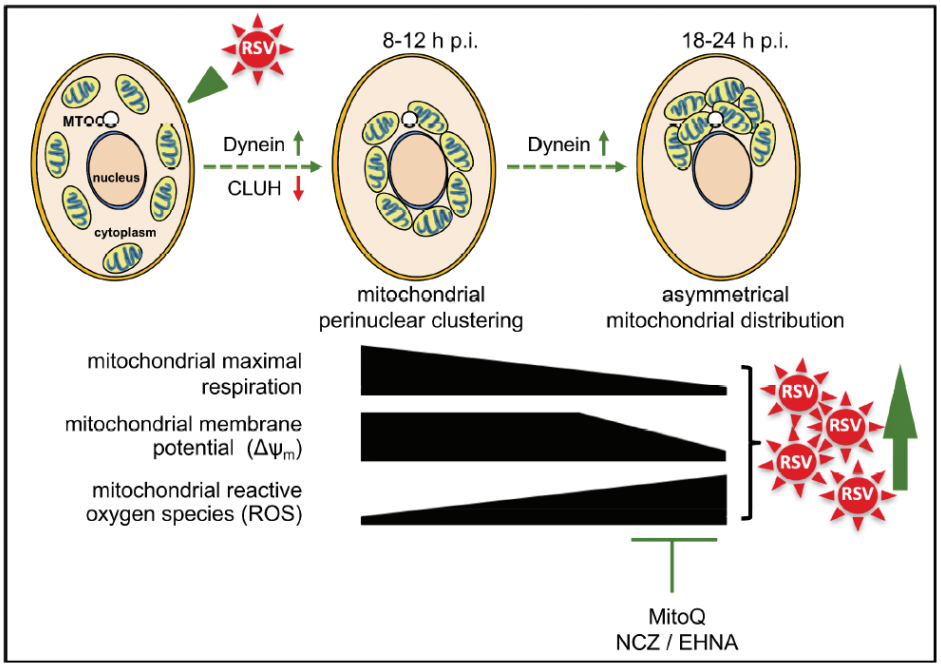

Figure 3. Schematic representation of the progressive host cell changes that favor RSV infection. RSV infection induces changes in mitochondrial organization, with mitochondrial perinuclear clustering early in infection (8 to 12 hours p.i.), followed by asymmetric distribution of mitochondria close to the MTOC later in infection (18 to 24 hours p.i.). Both phases of mitochondrial redistribution (top) are dependent on dynein components (inhibited by siRNAs directed at DYNLT1 or DYNC1H1), with perinuclear clustering limited by CLUH (siRNA directed at CLUH increases perinuclear clustering, as well as mitochondrial ROS production and RSV virus production). Accompanying these changes, RSV infection inhibits host mitochondrial respiration, disrupts maintenance of mitochondrial membrane potential (Dym), and enhances mitochondrial ROS generation. These events favor RSV infection as indicated, as RSV infectious virus production is decreased by disrupting microtubule organization using nocodazole (NCZ), by inhibiting the dynein-motor with EHNA, or using the mitochondrially targeted antioxidant MitoQ.

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