Visualising the Interplay Between Coronavirus replication and the Cellular Hypoxic Response

Peter A.C. Wing[1], Chidinma Okolo[3], Archana Jadhav[3], Nina Vyas[3], Kamal L. Nahas[3], Maria Harkiolaki[3], Jane A. McKeating[1,2]

[1]CAMS-Oxford Institute, University of Oxford.
[2]Nuffield Department of Medicine, University of Oxford.
[3]Beamline B24, Diamond Light Source.

Coronaviruses (CoVs) are globally relevant pathogens affecting humans and a diverse range of vertebrates. These viruses possess one of the largest documented RNA genomes within the viral kingdom and replicate in highly organised sub-structures in the cytoplasm known as 'double membrane vesicles' (DMVs); allowing replication to occur unhindered by cellular innate sensing. Key questions remain regarding the dynamics of DMV genesis and manipulation of cellular factors/pathways to achieve replication complex formation. Insights into these fundamental processes will help identify novel strategies to combat these viruses and strengthen our knowledge of coronavirus biology.

The cellular microenvironment is in constant flux, and to maintain homeostatic balance, cells must rapidly adapt to variations in their local surroundings. Given the fundamental nature of oxygen sensing, it is unsurprising that manipulation of these pathways impacts viral replication. Our recent work showed that activating HIFs through hypoxia or targeting prolyl hydroxylase enzymes with licenced inhibitors restricts respiratory pathogens such as respiratory syncytial virus, SARS-CoV-2, and seasonal coronaviruses. We further demonstrated that this antiviral activity in the replicative phase of the SARS-CoV-2 life cycle is dependent on the transcriptional activity of HIF-1α. To gain a deeper understanding of how HIF signalling limits SARS-CoV-2 RNA replication, we employed state-of-the-art structural imaging techniques to characterise the sub-cellular location of viral RNAs in hypoxic cells. Using single-molecule fluorescent-*in-situ*-hybridisation to quantify viral RNAs, we showed a substantial reduction in viral replication factories in hypoxic cells. To obtain a more in-depth analysis we employed cryo-soft X-ray tomography to visualise ultrastructural changes in SARS-CoV-2 infected cells. We discovered a spatial association between DMVs and mitochondria in infected cells under normoxic conditions, with viral replication complexes surrounded by filamentous mitochondria. Under hypoxic conditions mitochondria were observed to possess an abnormal elongated morphology coupled with a predominance of stalled viral replication complexes in infected cells. Intriguingly, transcriptomic analysis of SARS-CoV-2 infected Calu-3 cells cultured in low oxygen showed a reduction in HIF-target gene expression. Preliminary data indicate that SARS-CoV-2 infection limits nuclear translocation of HIF-1α in hypoxia thereby restricting HIF-driven gene expression. These findings provide new insights into the interplay between the cellular hypoxic response and SARS-CoV-2 replication, potentially identifying novel strategies to combat these viruses.