HIF signalling enhances innate immune control of Pneumovirus Replication

Jiyeon Ha[1], Sammi Ta[2], Parul Sharma[3], Xiaodong Zhuang[2], Simon de Neck[4], David Matthews[5], Peter Balfe[2], Jan Rehwinkel[6], Anja Kipar[3,4], James P Stewart[3], Peter AC Wing[1*] and Jane A McKeating[1,2*]

*Co-senior authors

1. CAMS-Oxford Institute, Nuffield Department of Medicine, University of Oxford, UK.
2. Nuffield Department of Medicine, University of Oxford, UK.
3. Department of Infection Biology & Microbiomes, Veterinary and Ecological Sciences, University of Liverpool, UK.
4. Laboratory for Animal Model Pathology, Institute of Veterinary Pathology, University of Zurich, Switzerland.
5. School of Cellular and Molecular Medicine, Faculty of Life Sciences, University of Bristol, UK.
6. Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.

Pneumoviruses cause lower respiratory tract infections, such as bronchiolitis or pneumonia in a range of mammalian hosts. Notable members include Respiratory Syncytial Virus (RSV) and Pneumonia Virus of Mice (PVM). RSV represents a global health burden in infants and the elderly with limited therapeutic options. During RSV-associated bronchiolitis, oxygen availability can diminish as lung epithelia experience progressive damage and aberrant inflammation, often necessitating oxygen support. Equally, the surge in metabolic activity during viral infection can further deplete oxygen levels within the microenvironment. Hypoxia inducible factors (HIFs) define oxygen-dependent cellular metabolic processes and regulate inflammatory responses in the lower respiratory tract. Our recent studies show a dual role for HIFs to restrict RSV and SARS-CoV-2 infection and associated inflammatory responses.

PVM is a natural pathogen of mice and induces bronchiolitis and pneumonia. To expand our observations to a physiologically relevant disease model we treated PVM infected mice with the prolyl hydroxylase domain inhibitor Daprodustat. We observed a significant reduction in viral antigen expression in bronchial and alveolar epithelial cells in the lung. Analysing pulmonary gene expression in the treated mice revealed a selective upregulation of several interferon-stimulated genes such as ISG15, IFNγ, IFNα-1 and -4, consistent with HIFs enhancing host innate sensing of PVM. We phenocopied these *in vivo* results using simple epithelial model systems and showed that PVM and RSV replication was attenuated by Daprodustat or low oxygen conditions (1% oxygen). Pneumoviruses replicate their genomes in subcellular compartments or inclusion bodies (IBs) that are formed by liquid-liquid phase separation. High-resolution imaging combined with single-molecule RNA FISH of RSV RNA showed that HIFs potently suppress IB formation. Next, we investigated whether the antiviral capacity of HIF was dependent on components of the innate viral RNA sensing machinery. RSV infection of Calu-3 cells genetically depleted for either RIG-I or MDA5 were no longer inhibited by Daprodustat treatment implicating HIF as a regulator of this signalling pathway. Collectively, our combined *in* vivo and *in vitro* studies show that HIF suppresses pneumovirus replication through enhancing innate recognition of viral infection. These findings highlight the significance of the hypoxia/immune signalling pathways in the control of respiratory viral infections.