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Fine mapping and characterization of the binding domain of the HRSV Phosphoprotein with the M2-1 protein

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study, we investigated the formation of PB1-F2 β -aggregates in IAV-infected cells at the single cell level using synchrotron radiation. Fourier-transform infrared (IR) and deep UV (DUV) microscopy are non-invasive techniques for monitoring biochemical changes *in situ* in cells and tissues. Human epithelial pulmonary cells (A549) and monocytic cells (U937) were infected with a wild-type IAV and its PB1-F2 knock-out mutant and harvested at different time post-infection. IR spectra were recorded in each condition and processed to evaluate the change in the component band of the spectra corresponding to the amide I (secondary structure) and the CH region (membranes). The data obtained were analyzed by component principal analysis and confirmed the presence of an IR specific β -aggregates signature only in IAV-infected cells expressing PB1-F2 in a cell- and time-dependent fashion. Taking advantage of the high frequency of tryptophan (Trp) residues in the sequence of PB1-F2, the increase of the auto-fluorescent signal of Trp recorded by DUV microscopy was correlated with the accumulation of the β -aggregates in IAV-infected cells. Furthermore, PB1-F2 compromises the integrity of the cellular membranes in a cell-type dependent manner. These data should provide further insight into the PB1-F2 structure-function relationship and help to decipher its role in the pathogenicity of the virus.

Disclosure of Interest: None declared

Poster 66

FINE MAPPING AND CHARACTERIZATION OF THE BINDING DOMAIN OF THE HRSV PHOSPHOPROTEIN WITH THE M2-1 PROTEIN

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Abstract: The RSV genome is transcribed into 10 mRNAs by the RNA-dependant RNA polymerase complex (RdRp). M2-1 protein is a transcription antiterminator which increases the processivity of the RdRp during transcription. M2-1 is recruited to RNA transcription sites by the phosphoprotein P. Since protein-protein interactions are a target for antiviral compounds, our objective is to obtain the crystallographic structure of the M2-1—P complex. The atomic structure of full-length tetrameric M2-1 is now available. However, since P is a naturally disordered protein, it is not possible to use full-length P for that purpose. The aim of this work was to finely characterise the M2-1 binding domain of P and to use this domain for co-crystallization trials. The M2-1-binding domain of P was previously mapped to residues 100-120 by internal deletions by Mason et al. By using NMR, we identified P residues ~ 90-100 as a region interacting with M2-1. Using recombinant proteins and deletions, the M2-1 binding site was finely mapped to amino acid residues 93-110. The role of amino acid residues in M2-1—P interaction was investigated by site-directed mutagenesis and pull-down assays, and the impact of these mutations on viral transcription was evaluated *in cellula* using an RSV minigenome. The results highlighted the critical role of some residues located in this region. The role of P oligomerization for M2-1—P interaction was also investigated.

Disclosure of Interest: None declared

Poster 67

NMR STUDY OF THE STRUCTURE AND INTERACTIONS OF THE HUMAN RESPIRATORY SYNCYTIAL VIRUS PHOSPHOPROTEIN

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Abstract: NMR allows to study the structure, dynamics and interaction properties of globular proteins, but also intrinsically disordered proteins and regions, which are not accessible to other structural techniques. Here we are focusing on the phosphoprotein (P), which is the main polymerase co-factor and necessary for both viral transcription and replication. As compared to other P proteins of *Mononegavirales*, hRSV P is rather short and does not comprise domains with stable tertiary fold outside the central trypsin-resistant tetramerization domain. Sequence analysis of P predicts the presence of a helical oligomerization domain and large disordered N- and C-terminal extensions. This domain arrangement is confirmed by NMR. Moreover we used backbone chemical shift analysis and ¹⁵N relaxation experiments to show that transient helices are formed in the N- and C-termini of P. At the C-terminus, nearly completely formed helices seem to prolong the oligomerization domain. At the N-