Unlocking Spatially-Resolved Transcriptomic and Proteomic Secrets of Century-Old Lungs from the 1918 'Spanish' Influenza Pandemic

Lauren E Steele[1], Chin Wee Tan[2,3,4] , Keng Yih Chew[1], James Monkman[2],, Sebastien Calvignac-Spencer[5,6], Thomas Schnalke[7], Navena Widulin[7], Habib Sadeghirad[2], Alyssa Rosenbloom[8], Brian Filanoski[8], Christine Kang[8], Kyla Teplitz[8], Murat Kekic[9], Eduard Winter[10], Joseph Beecham[8], Arutha Kulasinghe[2*], **Kirsty R Short[1*]**,

[1]School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, QLD, Australia
[2]Frazer Institute, Faculty of Medicine, The University of Queensland, Brisbane, QLD, Australia
[3]Division of Bioinformatics, Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia
[4]Department of Medical Biology, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, VIC, Australia
[5]Department of Pathogen Evolution, Helmholtz Institute for One Health, Greifswald, Germany
[6]Faculty of Mathematics and Natural Sciences, University of Greifswald, Greifswald, Germany
[7]Berlin Museum of Medical History at the Charite, Berlin, Germany
[8]Nanostring Technologies, Inc, Seattle, WA, USA
[9]The Ainsworth Interactive Collection of Medical Pathology, The University of Sydney, Sydney, NSW, Australia
[10]Natural History Museum Vienna, Vienna, Austria
*Contributed equally to this work

BACKGROUND

- The 1918 H1N1 influenza A pandemic was the deadliest influenza pandemic in recorded history, with an unusual peak in mortality in otherwise healthy young adults[1]
- Severe disease in otherwise healthy young adults was not observed in the subsequent influenza pandemics of 1957, 1968 and 2009[1]
- Can spatial transcriptomics and proteomics be used on historical samples to determine what was unique about the immune response to the 1918 influenza virus in young adults?

METHODS

Here, we used unbiased cutting-edge spatially resolved whole transcriptome (18,000 genes) and spatial proteomic atlas (570-plex) by the Nanostring GeoMx Digital Spatial Profiler to profile the lung architecture from 17 and 18 yr-old patients infected with the 1918 pandemic virus[2]

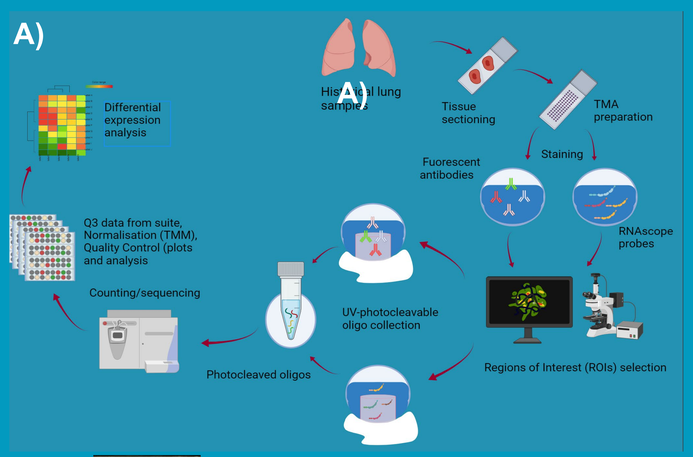

Figure 1: Process and tissue used for spatial transcriptomics and proteomics. A) Method overview of spatial transcriptomics and proteomics. Image created with Biorender. **B)** Historical lung samples used for analysis. Samples were obtained (from left to right) from a 17yr-old female, a 17 yr-old male and a 18 yr-old male who died of 1918 pandemic influenza.

REFERENCES

1. Luk, J. *et al., Clin Infect Dis* **33, 1375** (2001)
2. Patrono, L.V. *et al.* . *Nat Commun* **13**, 2314 (2022).

RESULTS

Different regions of interest display different pathology

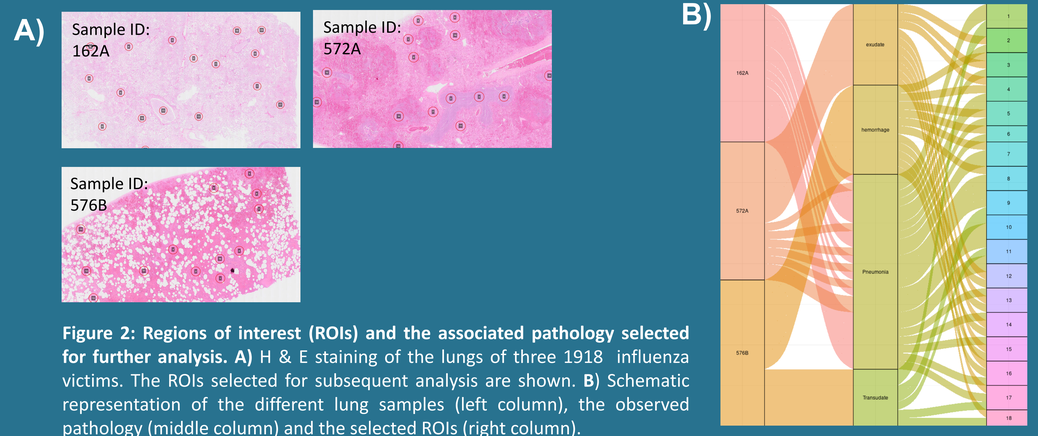

Figure 2: Regions of interest (ROIs) and the associated pathology selected for further analysis. A) H & E staining of the lungs of three 1918 influenza victims. The ROIs selected for subsequent analysis are shown. **B)** Schematic representation of the different lung samples (left column), the observed pathology (middle column) and the selected ROIs (right column).

Correlation between library size and nuclei count

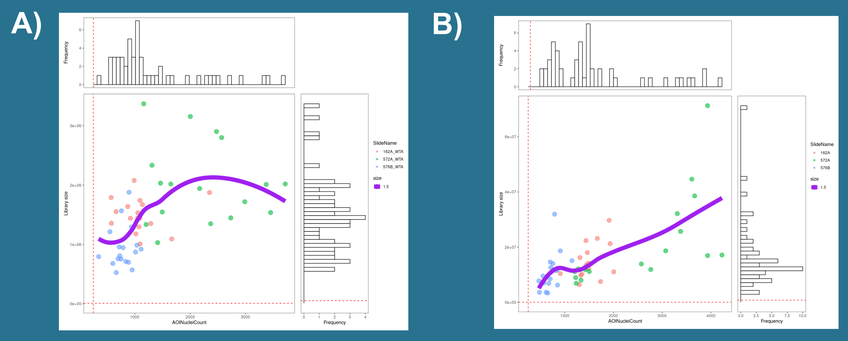

Figure 3: Quality control of spatial transcriptomic (A) and proteomic (B) analysis of 1918 lung samples.

Gene and protein expression is associated with pathology

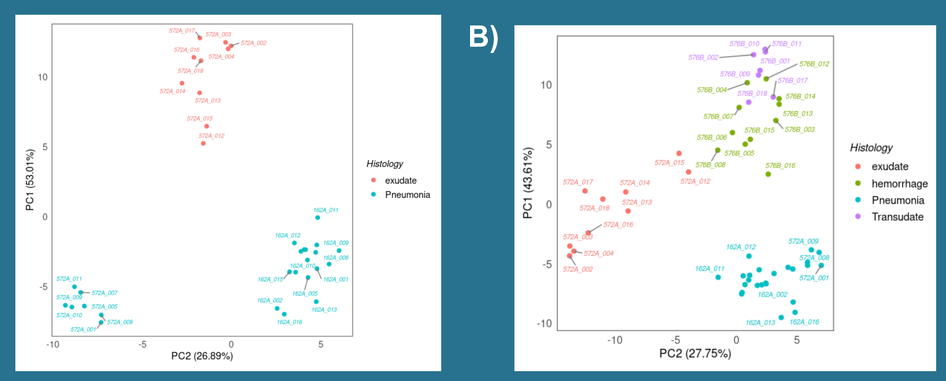

Figure 4: Principle component analysis of genes (A) and proteins (B) in regions of 1918 influenza virus infected lungs with different pathology.

Differential gene and protein expression between regions with different pathology

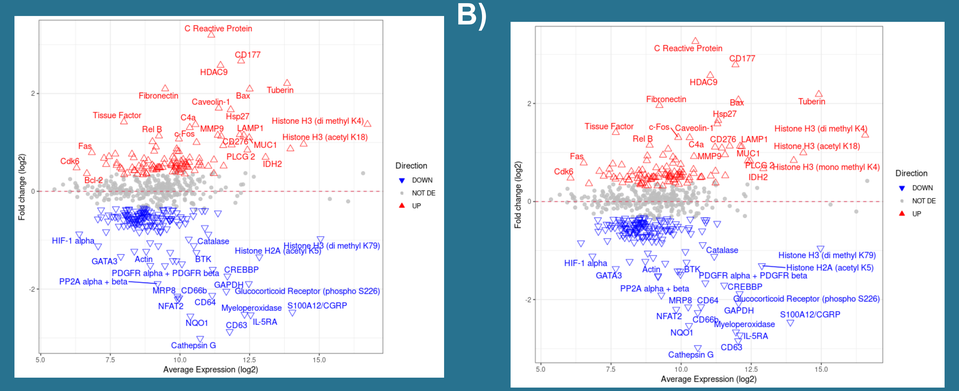

Figure 5: Differentially expressed genes (A) and proteins (B) in regions of pneumonia and exudate in 1918 influenza virus infected lungs.

CONCLUSIONS AND FUTURE DIRECTIONS

- Spatial transcriptomics and proteomics is possible on >100-year-old samples
- Next, we will analyse samples collected from young adults during the 1957, 1968 and 2009 influenza pandemics as well as uninfected patients
- Differentially expressed genes and proteins will be assessed to understand why young adults were uniquely susceptible to 1918 pandemic influenza
- This information plays an important role pandemic preparedness

A/Prof Kirsty Short, PhD
@K_RenfreeShort
k.short@uq.edu.au