Transcriptomic profiling of cardiac tissues from SARS-CoV-2 patients identifies DNA damage

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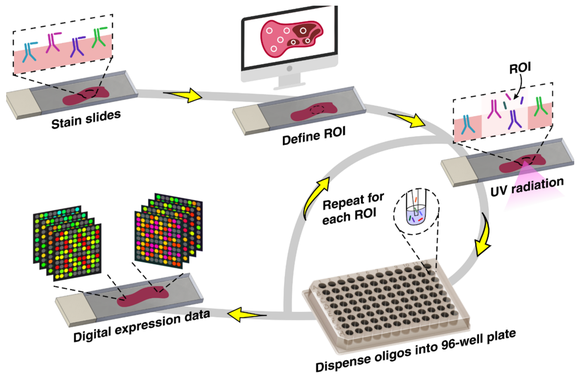
Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is known to present with pulmonary and extra-pulmonary organ complications. In comparison with the 2009 pandemic (pH1N1), SARS-CoV-2 infection is likely to lead to more severe disease, with multi-organ effects, including cardiovascular disease. SARS-CoV-2 has been associated with acute and long-term cardiovascular disease, but the molecular changes that govern this remain unknown. In this study, we investigated the host transcriptome landscape of cardiac tissues collected at rapid autopsy from seven SARS-CoV-2, two pH1N1, and six control patients using targeted spatial transcriptomics approaches. Although SARS-CoV-2 was not detected in cardiac tissue, host transcriptomics showed upregulation of genes associated with DNA damage and repair, heat shock, and M1-like macrophage infiltration in the cardiac tissues of COVID-19 patients. The DNA damage present in the SARS-CoV-2 patient samples, were further confirmed by γ-H2Ax immunohistochemistry. In comparison, pH1N1 showed upregulation of interferon-stimulated genes, in particular interferon and complement pathways, when compared with COVID-19 patients. These data demonstrate the emergence of distinct transcriptomic profiles in cardiac tissues of SARS-CoV-2 and pH1N1 influenza infection supporting the need for a greater understanding of the effects on extra-pulmonary organs, including the cardiovascular system of COVID-19 patients, to delineate the immunopathobiology of SARS-CoV-2 infection, and long term impact on health.

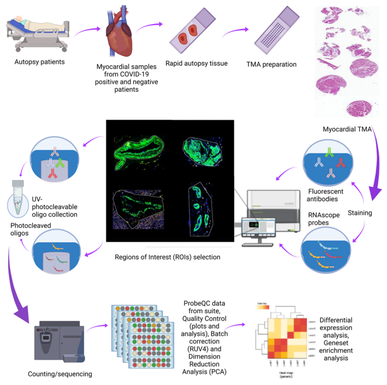
Methods

Myocardial tissues were obtained from patients at the Pontificia Universidade Catolica do Parana PUCPR in accordance with the National Commission for Research Ethics (CONEP) under ethics approval numbers: protocol number 3.944.734/2020 (for COVID-19 group, unvaccinated), and 2.550.445/2018 (for pH1N1 and control group). Families permitted the post-mortem biopsy of COVID-19 and H1N1pdm09 samples and conventional autopsy for the cases of the control group. All SARS-CoV-2 and pH1N1 patients were confirmed for infection by RT-qPCR of nasopharyngeal swab specimens. The control group did not have any indications of other infectious diseases. The study was ratified by The University of Queensland (UQ) Human Research Ethics Committee (HREC) .

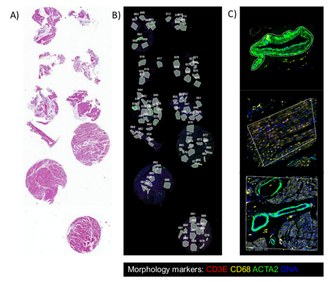


Tissue microarrays (TMA) were constructed of single cores from seven SARS-CoV-2, two pH1N1 and six control/healthy volunteer patients. A serial section TMA slide, was freshly sectioned and prepared according to the Nanostring GeoMX® Digital Spatial Profiler (DSP) slide preparation for RNA profiling (NanoString Technologies, GeoMx® Digital Spatial Profiler).

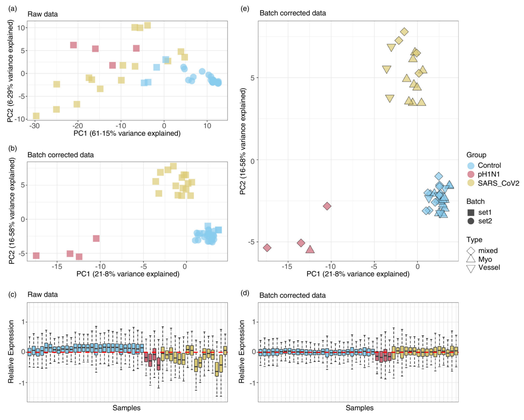
Results



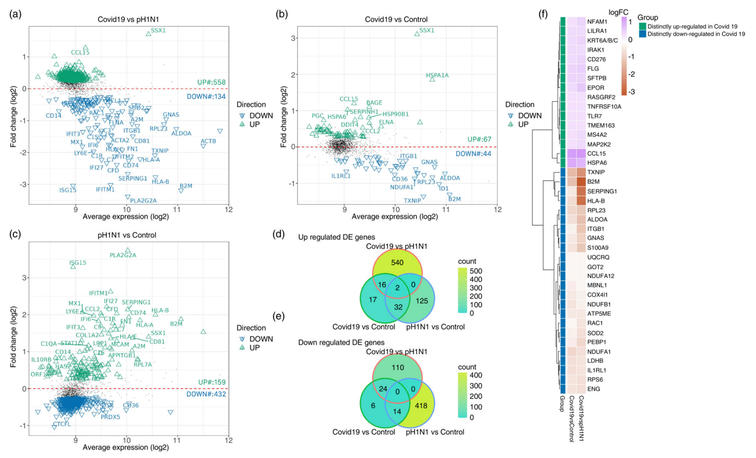
Cardiac tissues were collected from COVID-19 and pH1N1 patients at rapid autopsy. Samples were prepared onto tissue microarrays and profiled using targeted spatial transcriptomics (Immune Atlas Panel; NanoString technologies). Myocardial, blood vessels and mixed populations were captured using 'region of interest (ROI)' selection strategies to liberate the transcript data.



(A) Haematoxylin and eosin staining of the tissue microarray. (B) Regions of interest selected for spatial profiling by the Nanostring GeoMX digital spatial profiler (DSP) assay. (c) Regions of interest for the blood vessel (top), myocardium (middle), and mixed vessel/myocardium (bottom). Morphology markers for CD3E (red, T-cell marker), CD68 (yellow, macrophage marker) and ACTA2 (green, smooth muscle alpha-2 actin) and nuclear (blue) shown here.

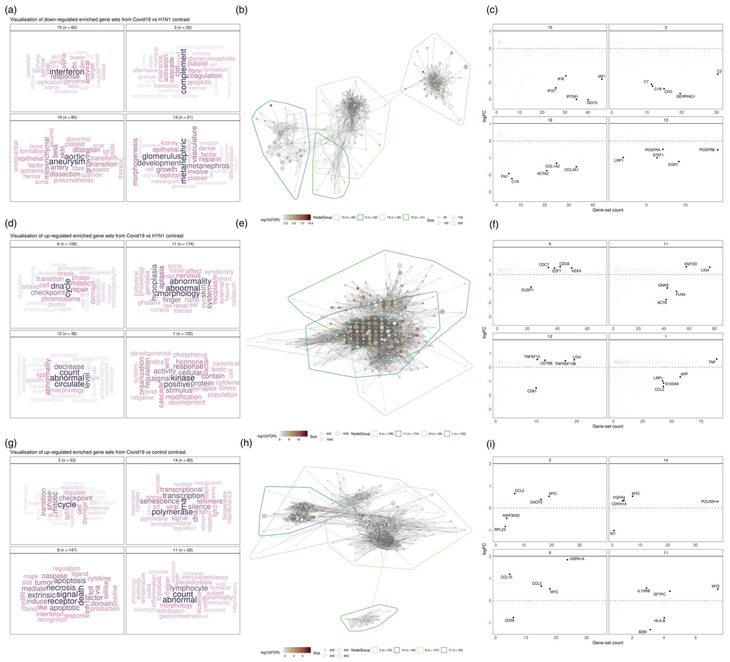


Batch correction and variability assessment of the spatial transcriptomic data. Principal component (PCs) analysis identifies the variability from batch effect in the transcriptomic data before (a) and after (b) batch correction. Relative log expression (RLE) plots (d and e) show the removal of technical variations after batch correction. Variabilities contributed by biological factors are visualized (c) in PC1 and PC2 of the batch-corrected data.



Differential expression analysis. Distribution of differentially expressed (DE) genes as a function of the average transcript expression (log2) and fold change (log2) identified in the following comparisons were visualized: (a) COVID-19 samples versus pH1N1 samples, (b) COVID-19 samples versus control samples and (c) pH1N1 samples versus control. Green triangles indicate upregulated, blue triangles downregulated, and black dots indicate non-DE genes. Differential expression genes were derived using voom-limma pipeline with limma: Duplication correlations and false discovery rate threshold with Benjamini–Hochberg adjusted $p < 0.05$. Venn diagram (d and e) is used to visualize the intersection of DE genes from each comparison. Heatmap (f) is used to show the fold change (log2) of the DE genes that are distinctly upregulated or downregulated in Covid-19 samples (the 16 and 24 genes showed in e).

Results



Visualization of significantly enriched gene sets different comparisons. (a, d and g) Cluster annotations based on text-mining analysis of gene set names. Nine gene set clusters representing biological themes of each comparison are displayed. (b, e and h) Gene set overlap graphs of gene sets enriched in up/downregulated DE genes in different comparisons with nodes representing gene sets and edges representing overlaps based on the Jaccard index. Nodes are coloured based on the significance of enrichment. (c, f and i) Fold change (log2-scaled) for genes belonging to gene sets in the cluster plot against the number of gene sets in the cluster the gene belongs to.

Conclusion

Key clusters of genes impacted were uniquely altered by SARS-CoV-2 infection and were distinct from pH1N1. These focus on DNA damage and repair pathways (confirmed with protein staining for gamma-H2AX) and the consequent cell cycle arrest pathways. Notably we observed upregulation of LIG4, an ATP-dependent DNA ligase which acts to repair DNA double-strand breaks via the non-homologous enjoining pathway. LIG4 expression is known to be enhanced following DNA damage and by Wnt/β-catenin signalling, suggesting that COVID-19-induced DNA damage might be responsible for induction of LIG4 in cardiac tissue. While this remains to be determined, the helicase NSP13 protein expressed by the related SARS-CoV-1 is known to induce DNA damage and replication fork stress by interacting directly with DNA polymerase *δ*. Given the NSP13 protein shares 99.8% sequence homology between SARS-CoV and SARS-CoV-2, it is possible that infection may induce DNA damage within myocardial tissue. However, SARS-CoV-2 infection has been observed, at least in vitro, to induce telomere shortening. This feature is attributed with senescence which aligns with the upregulation of this gene set pathway in COVID-19 myocardial tissues in our study. Interestingly, telomere stability is controlled by the DNA damage response proteins, as a telomere resembles a DNA break. Shortened telomeres result in a persistent DNA damage response, although at this point the function of these foci are unknown.

More comprehensive assessments of post-acute sequelae are needed to determine the short and long-term impacts of SARS-CoV-2 infection. It is known that DNA damage and impaired repair mechanisms foster genome instability and are involved in several chronic diseases. Long-term studies are needed to identify new onset heart disease from the early, and even subclinical, lesions as time post-infection transpires.