

Epigenetics of COVID-19 infected lung: systematic review and meta-analysis of gene expression data

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Abstract

Objective: To investigate homogeneity and efficiency of applying models of lung/bronchial organoids for SARS-COV2 infection research and evaluate the role of differentially expressed cytokine genes of interest.

Methods: in this systematic review and meta-analysis of Gene Expression omnibus datasets, studies of lung/bronchial organoids as models of SARS-COV2 infection were evaluated. 4 datasets of GSE160435, GSE148697, GSE150819, and GSE152060 were selected for our study. DESeq / EdgeR technique was used to identify Differential Expressed Genes (DEGs).

Results: The distribution of the pooled dataset showed small variations among the 4 selected datasets. K-means cluster analysis using the KEGG Pathway database revealed activation of a cluster of genes in response to coronavirus diseases including 51 genes in the pathway of KEGG, that could verify the organoids in comparison of real COVID-19 disease specimens. proinflammatory cytokines and Granulocyte-macrophage colony-stimulating factors were selected as our genes of interest-based on the literature. We only found significant upregulation of TNF-alpha, IL23A, and IL17A genes and significant Downregulation of CSF2RB, IL20RB/A, IL24B. while downregulation of CSF2 was in controversy with reported literature.

Conclusion: Based on the data that ultimately reached the conclusion of the interferon 1 function in COVID-19 pathology, this work may confirm the models of SARS-COV-2 infection in lung organoids; nevertheless, the contradiction to real-world studies requires more research.

Keywords: Granulocyte-macrophage colony-stimulating factor, COVID-19, SARS-COV-2, Organoid.

INTRODUCTION

Currently, limited evidence on the innate immune status of patients with SARS-COV-2 is available. COVID-19, the disease caused by SARS-COV-2 infection is a clinical condition that can range from minor symptoms to severe pneumonia, with respiratory failure, the need for mechanical ventilation, and death as a result (1). In addition, patients experiencing severe COVID-19 and requiring intensive care (ICU) has been known to have higher plasma levels of many intrinsic cytokines such as TNF α and IP-10, MCP-1, MIP-1A (2). These clinical features indicate the possibility of inflammatory conditions in the progression and severity of the disease. The most of lung damage in COVID-19 individuals may be caused by an increase in inflammatory cytokines such as interferon-, interleukin-6, and granulocyte-monocyte stimulating factor (GM-CSF) (3). Immunomodulation may help to reduce hyperinflammation and enhance clinical outcomes. Many cells, including macrophages and T-cells, produce GM-CSF in the lungs. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a key regulator of myeloid precursor proliferation and differentiation, as well as maturing granulocytes and mononuclear phagocyte function (4). Due to its function in both proinflammatory hypercytokinemia resulting in monocyte and macrophage activation, as well as antiviral immunity, GM-CSF is an immune-regulating cytokine that epitomizes the complexity and challenge of drug testing in COVID-19 (5). So, we aimed to investigate homogeneity and efficiency of applying models of lung/bronchial organoids

for SARS-COV2 infection research and evaluate the role of differentially expressed cytokine genes.

Methods:

In this meta-analysis study, Gene Expression omnibus (GEO) was systematically searched with keywords of SARS-COV2, COVID-19, “Lung”, and Organoid. Through the search results, records containing gene expression datasets of lung/bronchial organoids were selected. Inclusion criteria were databases containing human organoids of lungs that were infected by SARS-COV2. Studies had to publish read count raw data of the gene expressions to be included in our meta-analysis. Exclusion criteria were datasets that had evaluated a secondary treatment or medication on the organoids. Studies or datasets evaluating the human body specimens and biopsies were excluded. Based on this strategy, 4 datasets were selected for our study, shown in table 1.

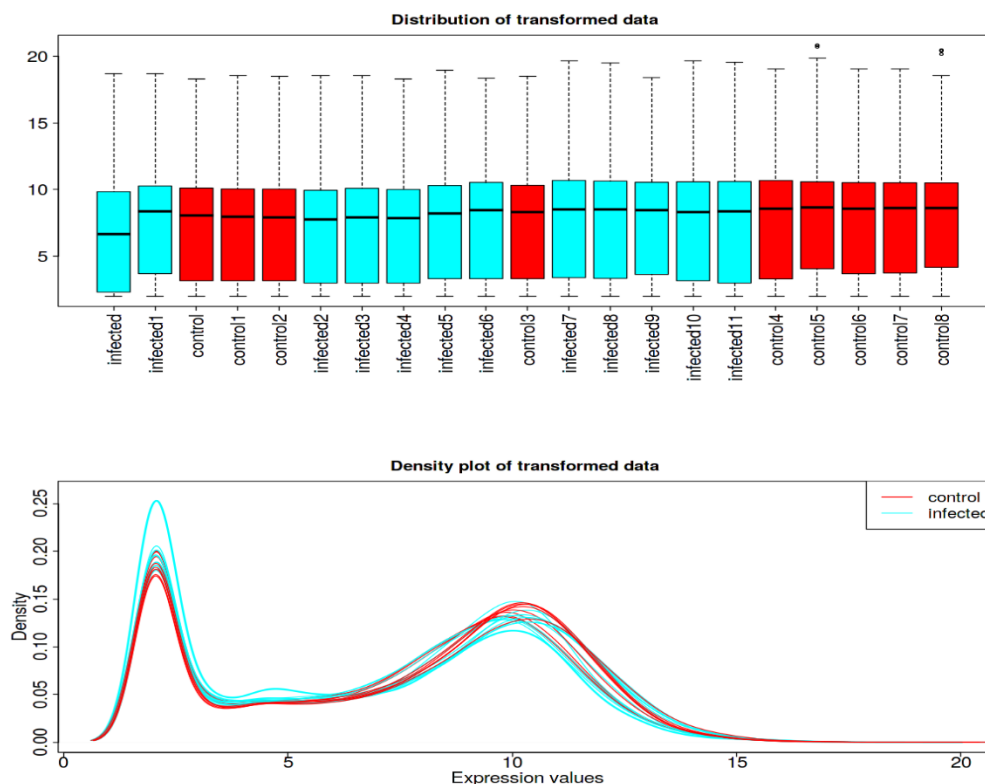
Table 1. included datasets in our study

Dataset id	author	Organoid model	platform
GSE160435	Changfu Yao	hESC-derived lung organoids	Illumina NovaSeq 6000
GSE148697	David Redmond	hPSC-derived lung organoids	Illumina NovaSeq 6000
GSE150819	Daisuke Okuzaki	human bronchial organoids (hBO), cryopreserved primary human bronchial epithelial cells (hBEpC)	Illumina NextSeq 500 and 6000
GSE152060	Liang Yang	Bronchial Organoids	Illumina NovaSeq 6000

Data was processed in excel software to match the gene ids of datasets by the VLOOKUP function. Finally, 33121 genes in 21 samples were evaluated. The final dataset was submitted to iDEP R-Shiny software (6) and The DESeq / EdgeR technique was utilized to determine differential expression analysis for each transcript. genes with minimal counts per million (CPM) were analyzed in EdgeR: $\log_2(\text{CPM}+c)$ at least a Minimum CPM of 0.5. Identifying Differentially Expressed Genes (DEGs) was conducted by DESeq2 method with FDR cutoff of 0.1 and Minimum fold change of 2.

As figure 1 shows, there were 12 samples of SARS-COV2 infected organoids and 9 samples of control untreated organoids. The distribution of the transformed data and density plot showed small variations among samples.

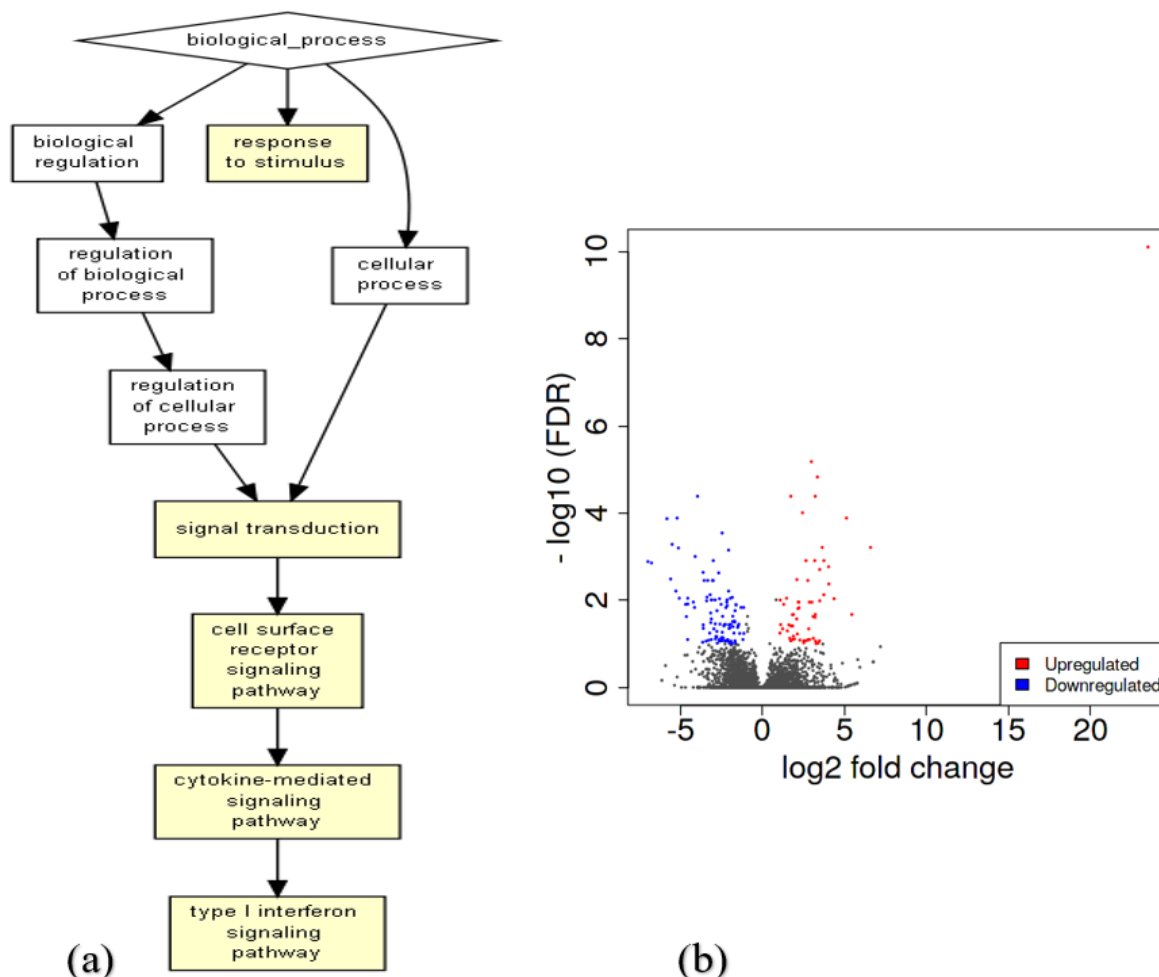
Figure 1. Data pre-processing of included databases



Results:

K-means cluster analysis using KEGG Pathway database revealed 4 different clusters in our dataset (figure 2). Enriched pathways in cluster C, contained coronavirus diseases including 51 genes in pathway. Using GO pathways enrichment in cystoscope-based analysis in online tools (7) with GO pathways with the significant difference in expression [signal transduction, cytokine-mediated signaling pathway, cell surface receptor signaling pathway, response to stimulus type I interferon signaling pathway] the activated pathway following the COSARS-COV-2 infection was visualized in Figure 1, a. Employing the DESeq2 tool with a false discovery rate (FDR) cutoff of 0.1 and a fold-change criterion of 2, 62 upregulated and 104 down-regulated genes were identified in infected vs. control samples (figure 2, b).

Figure 2. GO pathways enrichment (a) based on the differentially expressed genes (b)



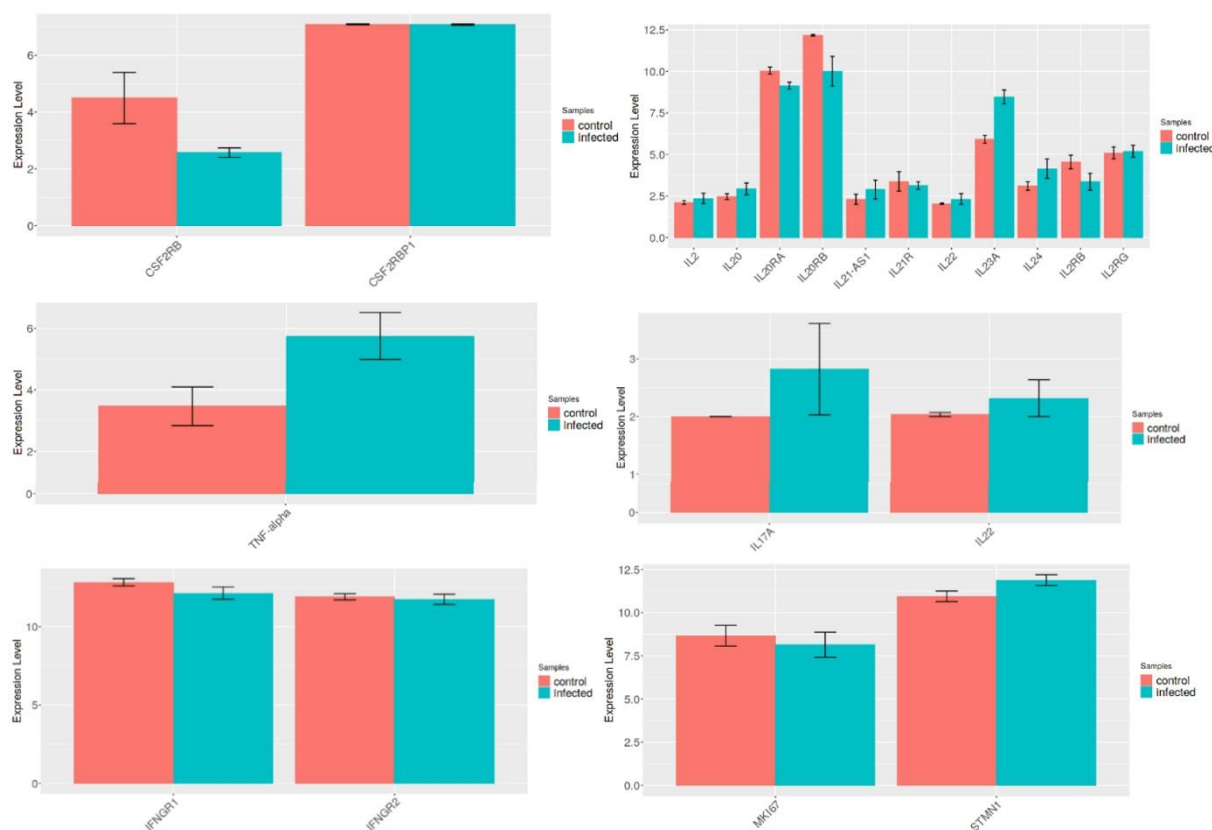
Upregulated genes were evaluated in GAGE analysis of infected vs. control replications where Type I interferon signaling pathway with 71 genes (P-value= 0.0019), Response to type I interferon with 76 genes (P-value= 0.0019), Response to virus with 285 genes (P-value= 0.0019), and Defense response to the virus with (P-value= 0.0066) were significant findings of the analysis. To evaluate T-cell activation by proinflammatory cytokines, through a literature review, as suggested by Zhao et al. (8) IL2, TNF-alpha, IL17A, IL17F, IFNG, and IL22 genes were compared between SARS-COV2 infected and control replicates. We only found significant upregulation of TNF-alpha, IL23A, and IL17A genes and significant Downregulation of CSF2RB, IL20RB/A, IL24B as shown in figure 3. Thirty top pathways are shown in table 3 with gene set size of minimum 15 and maximum 2000 and pathway significance cutoff (FDR) of =0.2.

Table 3. top 10 significant p-values and q-values for KEGG 2021 Human

Up-regulated pathways			Down-regulated pathways		
term	p-value	q-value	term	p-value	q-value
Hepatitis C	0.000011	0.000809	Retinol metabolism	0.000030	0.003375
Influenza A	0.000018	0.000809	Fatty acid degradation	0.001531	0.084955

Cytokine-cytokine receptor interaction	0.000043	0.001256	Serotonergic synapse	0.003083	0.101291
Rheumatoid arthritis	0.003304	0.048762	Arachidonic acid metabolism	0.004167	0.101291
Viral protein interaction with cytokine and cytokine receptor	0.004052	0.048762	Basal cell carcinoma	0.004563	0.101291
Human papillomavirus infection	0.004136	0.048762	Metabolism of xenobiotics by cytochrome P450	0.007687	0.142204
Amoebiasis	0.004283	0.048762	Staphylococcus aureus infection	0.014081	0.204395
NF-kappa B signaling pathway	0.004522	0.048762	Tyrosine metabolism	0.015590	0.204395
Lipid and atherosclerosis	0.004987	0.048762	Melanogenesis	0.016573	0.204395
Coronavirus disease	0.006508	0.057271	Drug metabolism	0.019772	0.219472

Figure 3. expression levels of genes of interest



Discussion

Organoids are a miniature, simplified version of a human organ. These Organoids originate from one or more cells - embryonic stem cells or induced multipotent stem cells - that can organize themselves in three-dimensional culture media. These three-dimensional models of human tissue can be used to test drugs before they are tested on humans. The application of organoids in COVID-19 research have not been evaluated previously. hESC-derived lung organoids, human bronchial organoids (hBO), cryopreserved primary human bronchial epithelial cells (hBEpC), and Bronchial Organoids are being used as models of SARS-

COV-2 infection, as stated in table 1. Scientists employed primary human lung epithelial 5 infection models to better understand the responses of the proximal and distal lung epithelium to SARS-CoV-2 infection. SARS-CoV-2 infected readily differentiated air-liquid interface cultures of proximal airway epithelium and 3D organoid cultures of alveolar epithelium, resulting in epithelial cell-autonomous proinflammatory response (9). In one of our utilized datasets(GSE165200), organoids of primary airway cells or pneumocytes were infected with SARS-CoV-2 to create in vitro COVID-19 lung models (10).

To evaluate the accuracy of these organoids for COVID-19 research, we compared identified DEGs and pathways of SARS-CoV-2 infected organoids with real world data of COVID-19 patients. Our in-silico analysis showed that many pathways being activated by this infection could finally come up to type I interferon signaling (11). The first interferon known to be released in response to viral infections is type I interferon. The interferon type I responses and its downstream cascade, which reach their peak in regulating viral replication and eliciting an acquired immune response, are crucial for the efficient innate immune response to viral infections. The key factor causing lung dysfunction and adversely affecting the course of infection may be the influence of type 1 interferons on myeloid cells. It is believed that type 1 interferons penetrate neutrophils, monocytes, and macrophages as a result of the CoV-SARS or CoV-MERS infection (12). An increase in these innate immune cells has serious consequences for the infected host, which in pulmonary immunopathogenesis manifests itself in conditions such as pneumonia and acute respiratory syndrome (13). The evidence is mounting that those with severe COVID-19 have a strong type I interferon response as compared to the delayed, perhaps inhibited response seen early in infection. Strong type I interferon response may worsen hyperinflammation in the development of severe COVID-19 through a number of different mechanisms (14). A greater understanding of the actions of type I interferon at various stages of infection and in patients with moderate vs. severe COVID-19 will lead to information about the therapeutic use of type I interferon in COVID-19 patients (14).

Our study showed significant downregulation of CSF2RB or Colony Stimulating Factor 2 Receptor Subunit Beta gene. Interestingly, therapeutic blocking and recombinant delivery of GM-CSF have been evaluated for COVID-19 patients, and there is substantial evidence for GM-CSF targeting in COVID-19. The alpha and beta subunits of the GM-CSF receptor make up the receptor (15). Inhibiting GM-CSF may be safer than targeting IL-6 in terms of safety because pharmacodynamic reduction of C-reactive protein and fever may be less severe, making subsequent infection diagnosis easier (15). Drugs targeting GM-CSF or its receptor have demonstrated effectiveness in cohort studies, but substantial controlled trial evidence has been anxiously awaited.

Bronchoalveolar lavage fluid study of individuals with severe COVID-19 showed that memory Th17 cells, such as tissue-dwelling tissue, had increased GM-CSF (16). While our study showed downregulated GM-CSF receptor Subunit Beta, it should be evaluated in clinical studies.

Conclusion:

Our study might verify the models of SARS-COV-2 infection in organoids of the lung based on the findings that finally came to the conclusion of the interferon 1 role in COVID-19 pathology; while controversy to real-world studies suggests further studies.

Data Availability Statement

Datasets included in this study are available online as cited.

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