COVID-19 Cell-cell Communication Imputation based on Single-cell RNA-sequencing Data Reveals Novel Immune Signals

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Keywords: COVID-19, Scrna-Seq, Cell-Cell Communication.

Abstract: Since its outbreak, the COVID-19 global pandemic had become one of the most serious diseases existed in the human history. Millions of people had been infected, and the pandemic is currently affecting the whole world in various fields such as public health, economy, and society. As a result, a better understanding of such disease is imminently needed to effectively control the ongoing pandemic. Cell-cell communications regulated by ligand-receptor pairs are crucial in coordinating diverse gene expression pathways. In a previous study, researchers implemented the single-cell RNA-sequencing technology on samples collected from COVID patients and healthy controls to obtain their cell-level RNA expression profiles. In this study, we statistically analyzed scRNA-seq data from a COVID patient and a healthy control generated by the previous study, and compared various gene expression between the samples with packages Scanpy and CellPhoneDB. Various plots were created to provide a comprehensive representation and comparison between the samples about gene expressions. The results showed numerous distinctions between the two sample in the overall gene expression level, the expression level of several immune-related ligand-receptor pairs across different cell type pairs, and the expression level of specific types of gene in different cell types. This study provided computational and statistical evidences related to COVID-19 pathology, which can be further pursued through biological experiments to obtain a better understanding of the global pandemic. The statistical analysis method used in this study showed an alternative way that can be potentially used to better understand the SARS-CoV-2 virus.

1 INTRODUCTION

On 31 December 2019, a novel coronavirus disease (COVID-19) was first reported in Wuhan city, China (Li 2020). As time passed by, more than 80,000 cases had been found from more than 30 provinces in People's Republic of China, and thousands of people around the world were died from such disease (Li 2020). The genes in ORF1 downstream region enables COVID-19 virus to replicate itself, forming nucleocapsid and glycoprotein spikes that allows the viruses to attach and enter the host cells (Shereen 2020). After successfully entered the host's cell, the SARS-CoV-2 will release and translate the genome RNA into pp1a and pp1ab, which are viral replicase polyproteins, and finally turn to viral proteins due to subgenomic mRNAs produced through discontinuous transcription (Shereen 2020).

Single-cell RNA-sequencing (scRNA-seq) is a technology that solves the long-existing challenge of using genotypes to infer the phenotypes, and therefore it can be used to obtain a better understanding of the dynamics of the organism's tissues and the complex relationships between diverse cell types (Hwang 2018). The technology enables researchers to establish valuable insights by examining information such as the population distribution of cells and regulatory relationship between genes (Hwang 2018). However, since the technology is still new, certain challenges exist and the technology can be furtherly improved. For example, currently it is hard to distinguish the 0 values in the data as either undetected or unexpressed, and the current clustering of cells may be conceiving due to the lack of reliable reference systems (Lähnemann 2020). While further improvement of the technology may bring deeper insights, the current scRNA-seq technology can still provide valuable information that can help researchers learn about cell-cell communications through analysis and examination of databases (Jin 2021).

Li, D.
COVID-19 Cell-cell Communication Imputation based on Single-cell RNA-Sequencing Data Reveals Novel Immune Signals.
DOI: 10.5220/0011312700003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 889-896
ISBN: 978-989-758-595-1

Cell-cell communication regulated by ligand-receptor complex plays a critical role in coordinating various biological processes such as the development and death of cells (Jin 2021). By analyzing the gene expression information obtained through scRNA-seq, such intercellular communications can be inferred to establish diverse biological discoveries (Jin 2021). CellPhoneDB (Efremova 2020) is a Python package that was developed to statistically analyze the database generated by the scRNA-seq. CellPhoneDB uses permutation tests to create null distributions that maps ligand-receptor interactions to understand cellular behaviors and responses to neighboring cells (Efremova 2020). With the analysis between ligand-receptor pairs in different cells in each given database, a better understanding of cell-cell communication network can be constructed with the detailed visual presentations in the Python package CellphoneDB. In this study, we developed a pipeline that utilized the CellphoneDB to find cell-cell communication patterns and discover biologically meaningful signals out of COVID scRNA-seq data.

2 METHOD

In a recent study, researchers collected heart, kidney, and lung tissue specimens from 19 individuals died from COVID-19 and 7 control at New York Presbyterian Hospital and Columbia University Medical Center. They then analyzed the samples using single-nucleus RNA sequencing and generated the gene expression matrices. The data was filtered and normalized to remove the background noise and control the quality of the data (Li 2020). More details including the filtering and normalization methods can be found in their paper. One COVID patient (l07) and one normal control (c52) counts and meta data generated through the experiment are further analyzed with two Python packages Scanpy and CellPhoneDB.

Firstly, to draw the box plots that show the percentage in total counts within a cell for the top 20 genes with the highest gene expression, we applied the function pl.highest_expr_genes in the Python package Scanpy. Then, to draw the violin plot that shows the number of genes expressed in each cell and the total counts per cell, we applied the pl.violin function in Scanpy. Next, we applied the pl.scatter function to draw scatter plots where the x-axis represents the total counts of expressed genes in each cell and the y-axis represents the number of genes expressed in each cell. The x-axis was specified as total_counts, and the y-axis was specified as n_genes_by_counts. Finally, to create violin plots that compare the expression levels of some highly variable genes across each different cell type between this two conditions, we again implemented the pl.violin function.

To obtain a more thorough understanding, we also used CellphoneDB (Efremova 2020) to create dot plots and heatmaps to describe the cell-cell communications and interactions between different ligand-receptor pairs in the samples. CellPhoneDB is a Python package that analyzes scRNA-seq data using the permutation test. The input data of the package should be scRNA-seq count data and an annotation of cell-types. A null distribution that describes the specificity between a ligand-receptor pair, which is represented by the average mean of expression level between ligand-receptor cell pairs, can be generated by randomly permuting clusters of all cell-types. The P value of the null distribution is based on the proportion of gene expression means that have as high or higher gene expression level than the actual mean. The specificity between a ligand-receptor pair can thus be inferred based on the overall amount of significant P values across the cell-type. Smaller P value means a more significant relativity between the ligand-receptor pair. In the end, different ligand-receptor pairs can be ranked based on their relativity, and the result can then be visually represented through functions that generate various graphs. To obtain the significant ligand-receptor pairs, we used the function statistical_analysis in CellphoneDB. Then, we applied the function dot plot in Cellphone DB to create a dot plot where the x axis represents the ligand-receptor cell types, and the y axis represents the ligand-receptor pairs in these cells. In the end, we used the function heatmap plot to create heatmaps showing the number of significant ligand-receptor pairs between each cell type pairs.

3 RESULTS

Through the data and plots obtained, it can be clearly observed that there are many differences between the heathy control and the COVID patient.

Table 1: Cell type proportions in the healthy control and the COVID patient.

Healthy Control		COVID Patient	
Cell Type	Proportion	Cell Type	Proportion
T cells	0.040762	T cells	0.040762
Myeloid	0.118742	Myeloid	0.118742
Epithelial cells	0.652193	Epithelial cells	0.652193
B cells	0.002215	B cells	0.002215

Fibroblasts	0.101019	Fibroblasts	0.101019
Neuronal cells	0.009304	Neuronal cells	0.009304
Mast cells	0.005760	Mast cells	0.005760
Endothelial cells	0.062915	Endothelial cells	0.062915
APC-like	0.007089	APC-like	0.007089

Table 1 represents the proportion of different types of cells in the sample of the healthy control and the COVID patient. In the healthy control, the proportion of Epithelial cells is the greatest, but in the COVID patient T cells are significantly more than in the healthy control. The pattern is the same for other immune cells like B cells and mast cells, which suggests the potential role that immune cells play in patients with COVID-19.

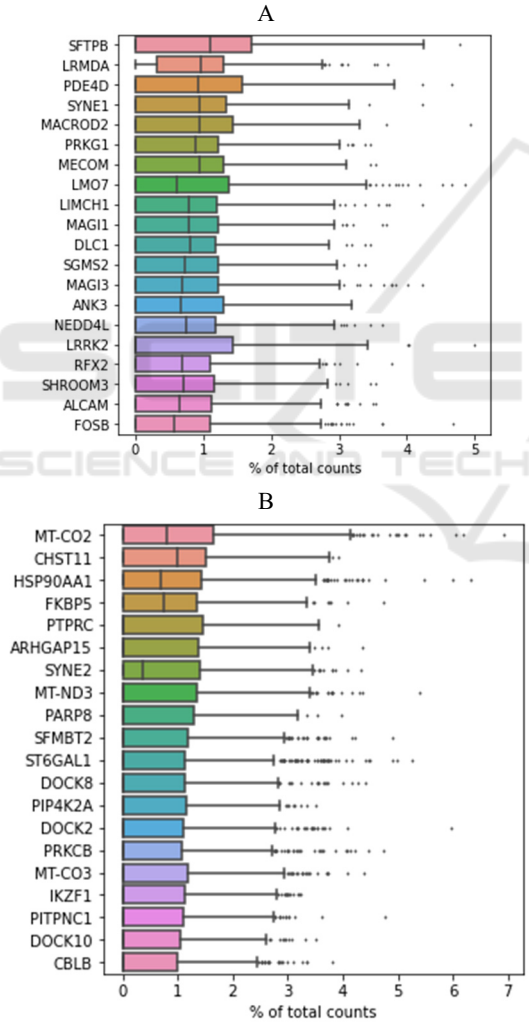


Figure 1: Box plots of highest expression genes in healthy control (A) and COVID patient (B).

Figure 1 are box plots that represent the percentage of different genes in each cell across all cells in the normal control and COVID patient. By comparing the two plots, it can be observed that the most enriched genes between the COVID patient and the healthy control are distinct. Many genes that were not enriched in the normal control turned out to be dominant in COVID patient. For example, CHST11 is more dominant in the COVID patient than in the normal control. Previous study had shown that the increase in expression of chondroitin sulfotransferases like CHST11 may Lead to COVID progression of respiratory disease (Bhattacharyya 2020). At the same time, FKBP5 in COVID patient is also more enriched than in normal control. FKBP5 has been known as an elite gene related to schizophrenia and depression, and the alteration in its expression is associated with autism, and this implies the potential impact that COVID-19 has on neuropsychiatric disorders (Melms 2021). Last but not least, the HSP90AA1 is also enriched in the COVID patients. HSP90AA1 is "a highly-conserved molecular chaperone protein" (Wauters 2021) and has been proved to be involved in wide ranges of virus infections and replications (Geller 2012). Previous study had shown that HSP90AA1 has positive correlation with the viral RNA and high level of expression in cells with SARS-CoV-2, also known as COVID-19, while the level is not high in SARS-CoV-1, which implies the potential role it plays in the COVID-19 viral infection progress (Wauters 2021, Wyler 2021).

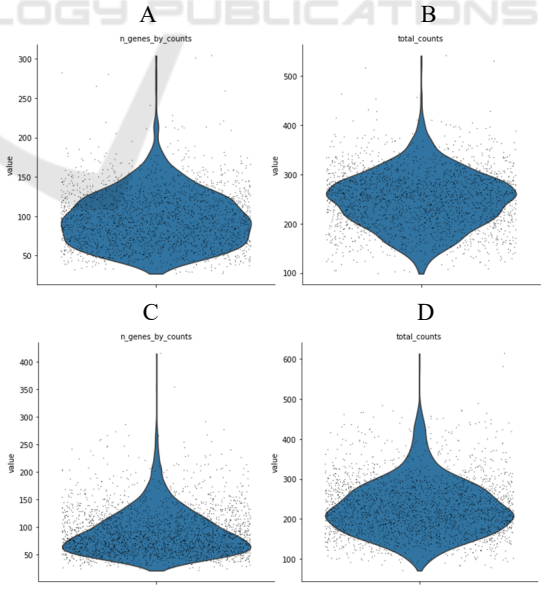


Figure 2: Total number of genes expressed and the gene expression level in each cell in normal control (A, B) and COVID patient (C, D).

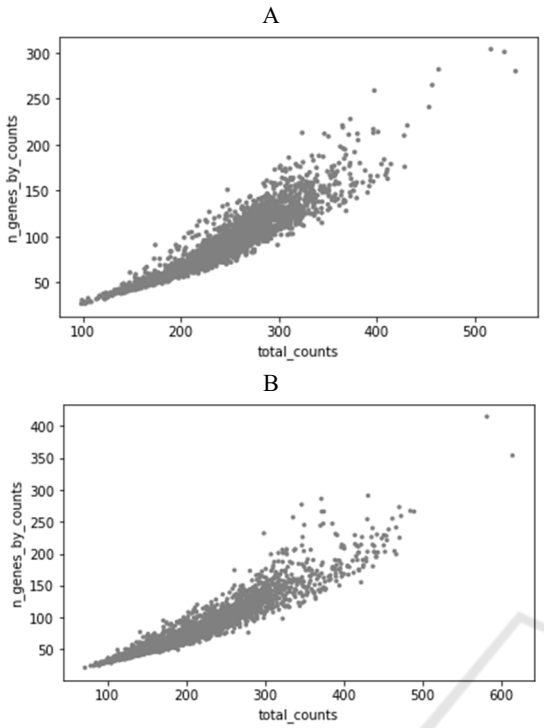


Figure 3: Scatter plot of gene expression in healthy control A and COVID patient B.

Figure 2 and figure 3 suggest overall suppression of gene expression across different cell types in the COVID patient. Figure 2A represents the total amount of expressed genes in cells of the normal control, and figure 2B represents the gene expression level in each cell of the COVID patient. By comparing the plot with that of the COVID patient, the suppression can be observed. Through the comparison of figure 2A and figure 2C, it can be observed that the average total amount of gene expressed in the patient is less than that of the healthy control. Meanwhile, by comparing figure 2B and figure 2D, the similar observation can also be made on the gene expression level in cells of the COVID patient. The scatter plots in figure 3 provides deeper insights of the observation. It clearly shows that there are higher extreme expressions in the COVID patient, where the outliers for figure 3B are more extreme than the ones in figure 3A. At the same time, the ranges of the distributions in figure 3B are also broader than in 3A.

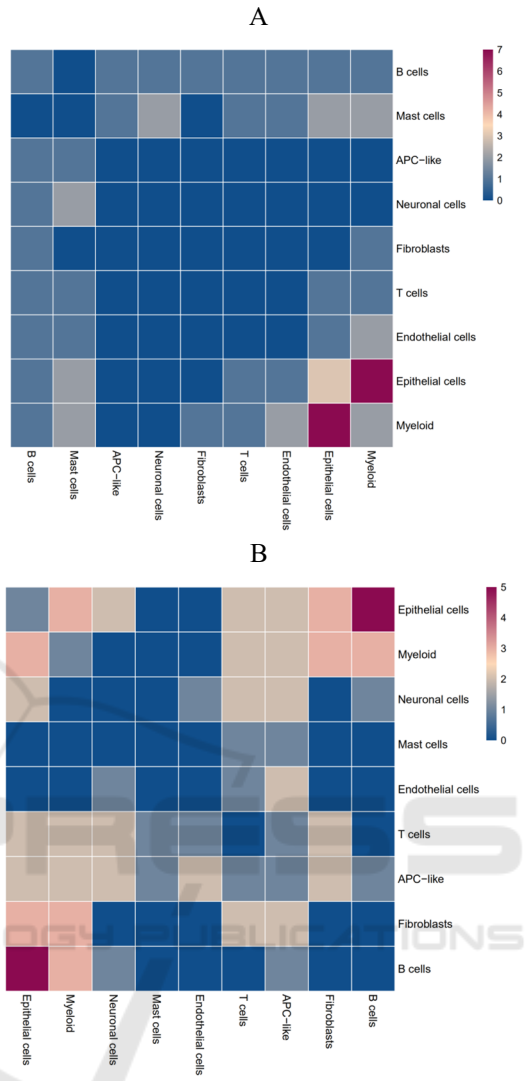


Figure 4: Heatmap plot of gene relativity between ligand-receptor cell pairs in healthy control A and COVID patient B.

Figure 4 represents the overall gene relativity between ligand-receptor cell pairs in the healthy control and COVID patient. Figure 4 is a heatmap plot, and the color in each square represents the gene relativity/number of significant ligand-receptor pairs between the corresponding ligand-receptor cell pairs. Due to the overall gene expression suppression showed by figure 2 and 3, the overall amount of significant ligand-receptor pairs decreased, which is why the maximum amount significant ligand-receptor cell pairs in figure 4B (5) is less than that of figure 4A (7). By comparing figure 4A and figure 4B, it can be clearly observed that the communications between Epithelial cells and B cells are more significant in the COVID patients, as the color

changed from light blue to red. Previous studies had shown that the airway epithelial-immune cells interactions can cause heightened harm to airway system, including lung injury, tissue inflammatory damage, and even respiratory failure (Chua 2020). This result further implies the potential role that B cells play in interacting with epithelial cells in patients with COVID 19. At the same time, the cell type relationships between B cells and Myeloid cells are also stronger in the COVID patients, as the color changed from gray to pink. Previous study has shown that the decreased interaction between Epithelial and myeloid may be caused by the depletion of epithelial cells upon COVID infection (Stanford 2012). This result implies the potential role that B cells play in interacting with Myeloid in COVID patients and adds new potential evidence to the previous study.

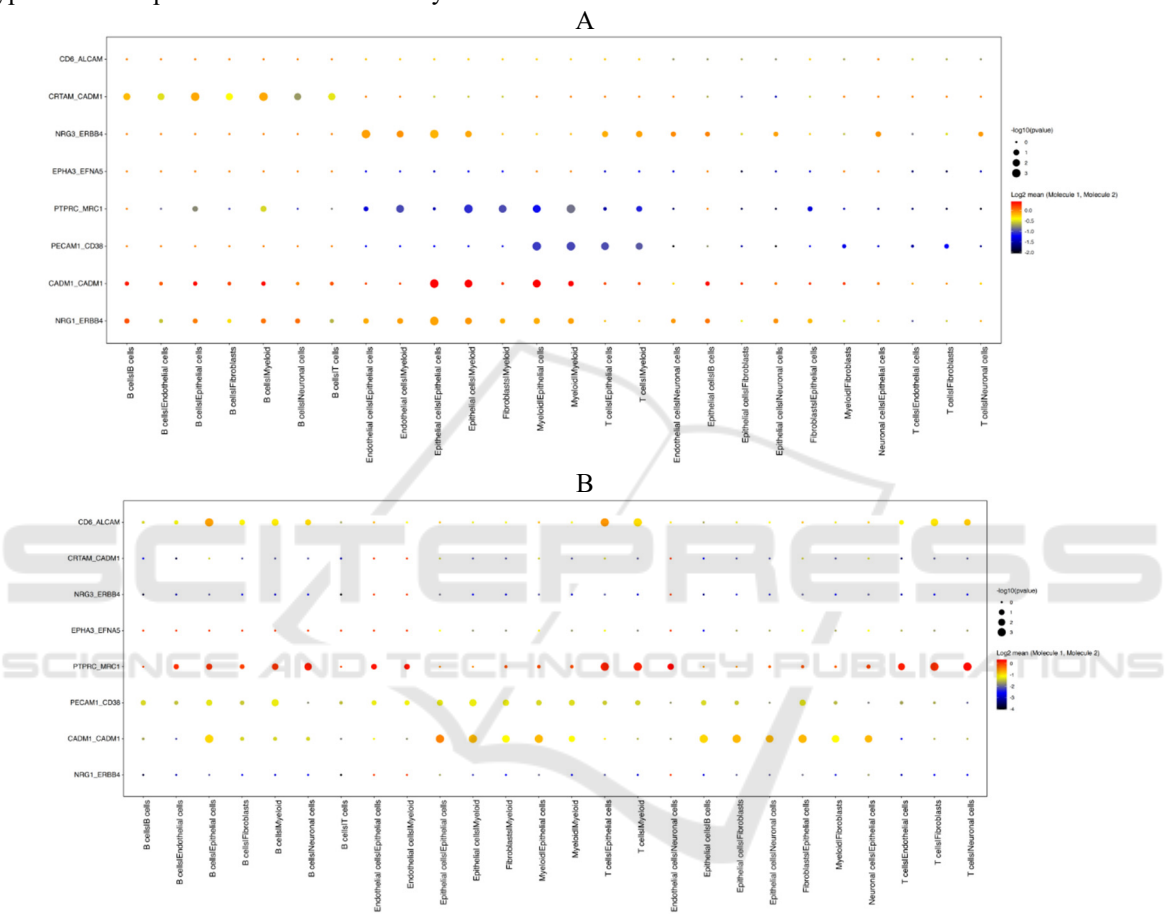
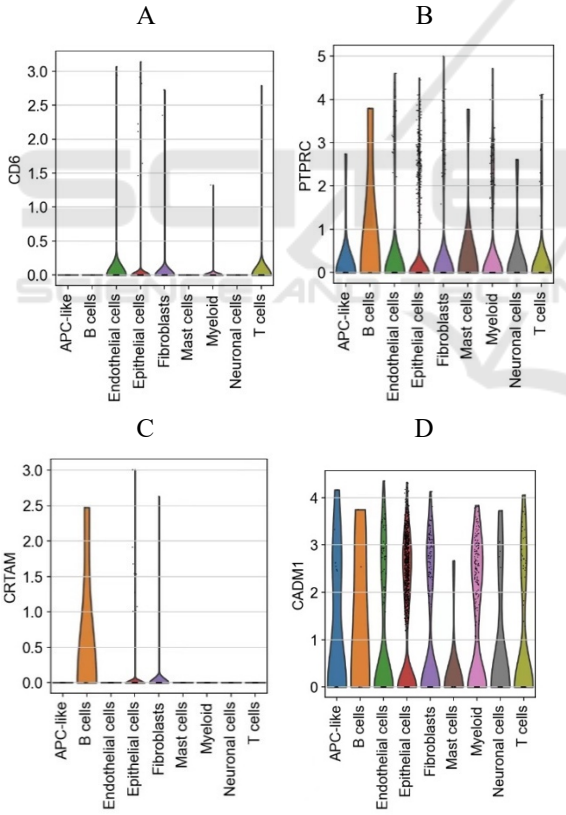


Figure 5: Dot plot of gene relativity between ligand-receptor cell pairs in healthy control A and COVID patient B.

Figure 5A and 5B provide further analysis of the gene relativity between ligand and receptor cells by representing specific gene pairs' interactions between different ligands-receptors pairs. The color of the dot shows the mean value of the expression level. The higher the expression level, the stronger the interaction is, and the color will be closer to red. The size of the dot represents the negative log p-value, and it shows how statistically significant the relationship is. The larger the dot means smaller the p value, which means more statistical significance of the result regarding the relativity between the ligand-receptor pair. By comparing figure 5A and 5B, it can be observed that there is significantly heightened relativity between PTPRC and MRC1 genes in the COVID patient. The large red dots in plot B shows significant interactions between B/T cells and. endothelial/epithelial/myloid/fibroblasts/neuronal cells. B cells and T cells are both immune cells, and the result shows the strong interaction within immune cells or between immune cells and non-immune cells with the PTPRC-MRC1 pathway. At the same time, CD6-ALCAM pathway also had strong expression between B/T immune cells and endothelial/epithelial/myloid/fibroblasts/neuronal cells in COVID patients. Previous studies showed

that CD6-ALCAM pathway is responsible for T cell activation and migration (Ampudia 2020). Through interacting with its ligand activated leukocyte cell adhesion molecule (ALCAM), CD6 promotes immune synapse formation (Ampudia 2020). The result shown in figure 5 provides further support to the previous study, pointing out the potential involvement of this pathway on B cells and non-immune cells in the COVID patients. Last but not least, the PECAM1-CD38 pathway also had stronger expression in the COVID patient compared to healthy control. PECAM1 is known as the ligand of CD38, and in a previous study the potential correlation between PECAM1 and CD38 expression in patients with B-cell chronic lymphocytic leukemia (B-CLL) was suggested (Ibrahim 2003). The result in figure 5 implies the potential role that PECAM1-CD38 gateway plays in patients with COVID 19, adding another topic to be further investigated besides the unsolved topic in the previous study.



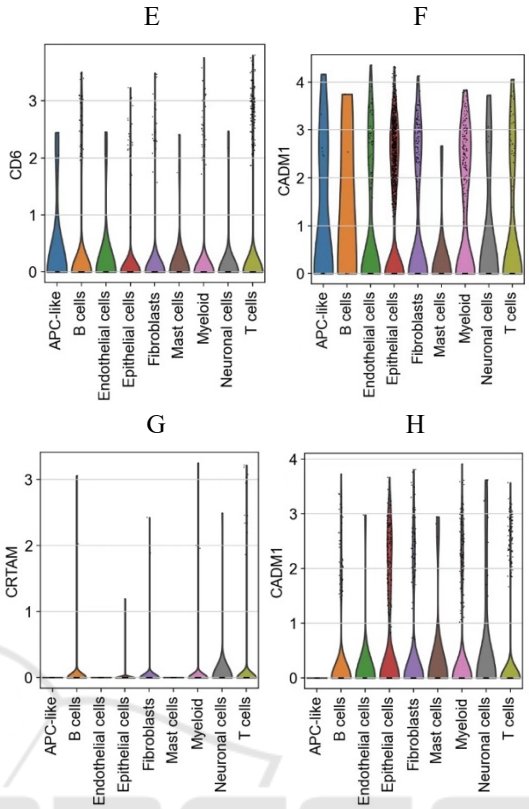


Figure 6: Violin plots that shows expression of different genes in different cells in healthy control (A,B,C,D) and COVID patient (E,F,G,H).

Figures on the top and figures on the bottom represent the expression of some significant genes in different cells of the normal control and the COVID patients. By comparing figure 6A and 6E, it can be observed that gene CD6 overall has higher expression in most type of cells in the COVID patient, especially for APC like cells, B cells. Both APC like cells and B cells are immune cells, and previous studies have shown the involvement of CD6 in regulating immune responses and in contacts between cells (Consuegra-Fernández 2018). The result in this study supports the previous result and implies the potential role it plays in COVID patients. At the same time, the comparison between figure 6B and 6F shows that PTPRC gene expression overall is significantly heightened in various cell types of the COVID patient. Previous studies have shown the important role that PTPRC plays in regulating the immune functions of B cells and T cells (Chen 2021, Shereen 2020). The result indicates altered immune functions in cell types such as T cells and B cells in COVID patients. In contrast, the expression level of CRTAM is significantly lower in COVID patients comparing to the normal control.

Previous studies had showed that CRTAM can generate cytotoxic T cells and clear viruses in mice (Kusnadi 2021). The result in figure 6 adds on to the previous study, implying that COVID may have disrupted the immune system in the COVID patients. Thus, the expression level of some immune related genes is down regulated in B cells. Moreover, the CADM1 gene in immune cells of the COVID patient is also significantly lower than in the healthy control. CADM1 helps adhesion of cell, delivering cell signals through contact, and plays an import role in establishing immune responses for immune cells by acting as the scaffolding molecule (Quincozes-Santos 2021, Sawada 2020). The result in this study shows the potential role that CADM1 plays in B cells as its expression largely decreased in the COVID patient.

4 CONCLUSIONS

This study statistically analyzes and compares the data obtained from the sample of the COVID patient and healthy control through single-cell RNA-sequencing. Using various statistical methods, results were generated to provide a comprehensive and detailed comparison between the gene expression of the healthy control and COVID patient. Through the comparison between the samples, many significant differences can be observed. While the gene that is responsible for major gene expression is different in the two samples, the overall gene expression and number of genes expressed decayed in the COVID patient. At the same time, genes have higher expression in immune cells like B cells and T cells of the COVID patient, and the communications between non-immune cells and immune cells also increased. All these are evidence of the immune cells' roles in different part of human body. By further analyzing the different gene pathways of ligand-receptor pairs, more detailed interactions and correlation were understood. The result also provided new topics that require further research to gain more insights. Since this study only analyzes two of samples, possible errors may exist due to the limit data. At the same time, direct corrections between genes and proteins produced are assumed in this study. The input of Cellphone DB (Efremova 2020) is mRNA-level data, but the conclusions are inferred based on protein interactions. This can be solved through further analysis of more samples, and the result obtained can be more accurate. However, the study can act as a starting point and provide a novel way to analyze data and better understand the effect of COVID-19 on patients.

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