

# Unveiling SARS-COV-2 associated Organ Specific Cell Types and Corresponding Functional Hubs

Ashmita Dey<sup>1</sup>, Sagnik Sen<sup>1</sup>, and Ujjwal Maulik<sup>1</sup>

Department of Computer Science and Engineering  
Jadavpur University, Kolkata, India  
[ashmitadey19@outlook.com](mailto:ashmitadey19@outlook.com)  
[sagnik.sen2008@gmail.com](mailto:sagnik.sen2008@gmail.com)  
[umaulik@cse.jdvu.ac.in](mailto:umaulik@cse.jdvu.ac.in)

**Abstract.** The novel coronavirus or 2019-nCoV is originated from Wuhan city, China and becomes pandemic. Studies showed that Angiotensin I converting enzyme 2 (ACE2) play an important role of host receptor by allowing the 2019-nCoV to enter the human body which thereafter causes final infection. ACE2 is highly expressed in Bladder, Ileum, Kidney, Liver and Lung compare to Lung tissue. In this study, the single-cell RNA-Seq of the five tissues from different humans are curated and cell-types with high expression level of ACE2 is identified. Subsequently the protein-protein interaction network is formed. The significant genes (AGT, PPARG, PPARA) responsible for designing the functional hubs are selected for the Gene Ontology analysis. Through the GO analysis, the biological involvement of the genes including ACE2 in 2019-nCoV infection is uncovered.

**Keywords:** 2019-nCoV · ACE2 · Single-cell RNA Seq · gene network · Gene Ontology.

## 1 Introduction

The outbreak of the recent pandemic COVID-19 has been declared as a public health emergency by World Health Organization. Interestingly, these viruses have shown a strong binding with a cell receptor, namely angiotensin-converting enzyme type II (ACE2) through the Spike S virulent protein. The study of Zhang et al. has provided the molecular mechanism of spike protein entry (Zhang, Penninger, Li, Zhong, & Slutsky, 2020). As per the study, Transmembrane proteases play a vital role by creating cleavage in ACE2 and activating ACE2 cell receptor. From Lung, the sharing significant expression levels of ACE2 and TMPRSS2 have been observed in Pulmonary Alveolar type II (PAT2) cells (Lukassen et al., 2020). Similarly, each of the vital organs viz., Kidney, Liver, Ileum, Bladder must have certain cell types which are sharing significant RNAseq expression of ACE2 and TMPRSS2. It is expected that these cell types are participating in nCoV infection are participating in nCoV infection. In a cellular condition, the

significant expressed ACE2 can modulate co-interacting functional hubs. Similarly, the rate of transmission of the SARS-CoV-2 must be associated with many more molecular members of different cell types. So far no studies has reported the involvement of proteomic samples associated with ACE2. The study of the functional hubs with neighboring proteomic samples can be a key point in terms of therapeutic possibilities fucntional dysregulating during the infection.

In this study, we have studied the single-cell RNAseq data for Lung, Ileum, Kidney, Bladder, and Liver. The organ specific cell types and corresponding markers are fetched based on the reported ACE2 and TMPRSS2 expressions in PAT2 cells. For each of the defined cell types, significant proteomic markers are shortlisted based on the communities protein-protein interaction network (PPIN) of cell specific significant transcripts. The functional hubs are identified applying k-means network clustering. Depending on the potential hubs from PPIN, Gene Ontology analysis is performed to understand the biological process involved in SARS-CoV-2.

## 2 Materials and Method

Publicly available ScRNASeq datasets of different tissues and organs (Bladder (Yu et al., 2019), Ileum (Wang et al., 2020), Kidney (Liao et al., 2020), Liver (MacParland, Liu, & McGilvray, 2015) and Lung (Reyfman et al., 2016)) from diverse human bodies are curated from GEO (<https://www.ncbi.nlm.nih.gov/geo/>). The detail information of the acquired datasets are as follows: Bladder, GEO Accession No. GSE129845 sample GSM3723358; Ileum, GEO Accession No. GSE134809 sample GSM3972018; Kidney, GEO Accession No. GSE131685, 3 healthy kidney tissues; Liver, GEO Accession No. GSE115469, 5 healthy human patients; Lung, GEO Accession No. GSE122960.

Seurat V3.0 (Stuart et al., 2019) is used to process the raw count matrix. To remove the unwanted cells from the dataset, cells less than 200 expressed genes and gene expression in less than three cells are eliminated. Moreover, cells are filtered with  $> 5\%$  mitochondrial counts. Then, the gene expression data is normalized by employing "logNormalize" method. Before cell clustering and dimension reduction process, a subset of features is calculated that contains 2000 features per dataset. This displays the high cell-to-cell variation with the resolution 0.5. Cells are clustered applying "FindClusters" function and obtained a graph-based structure in PCA space. These clustered cells are projected through the UMAP non-linear dimension reduction technique.

"FindMarkers" function is performed to identify differentially expressed genes of each cluster. The expression level of ACE2 is evaluated from each clusters and those clusters show high expression value are selected with the reference of ACE2 is highly expressed in lung AT2 cell-type. We interpret that any cell-type with positive ACE2 is exposed to 2019-nCoV infection. Accordingly, the corresponding organs are reported as highly risk.

### 3 Result and Discussion

The single-cell RNA-Seq data of different human organs revealed the information regarding the responsible cell-types. These vital organs can be subdivided into three classes based on their functionalities. Interestingly, most of the cell types associated with a significant expression value of ACE2 are also sharing significant expression value of AGT and PPAR family transcripts e.g., PPARA and PPARG. However, only those cell types have these significantly expressed samples where TMPRSS2 shows a higher expression. In Table 1, the organ-specific potential cell types and corresponding significant samples have mentioned. The highly significant samples are selected based on the functional hub which is strongly associated with ACE2.

#### 3.1 Cell Specific Functional Hubs from Lung

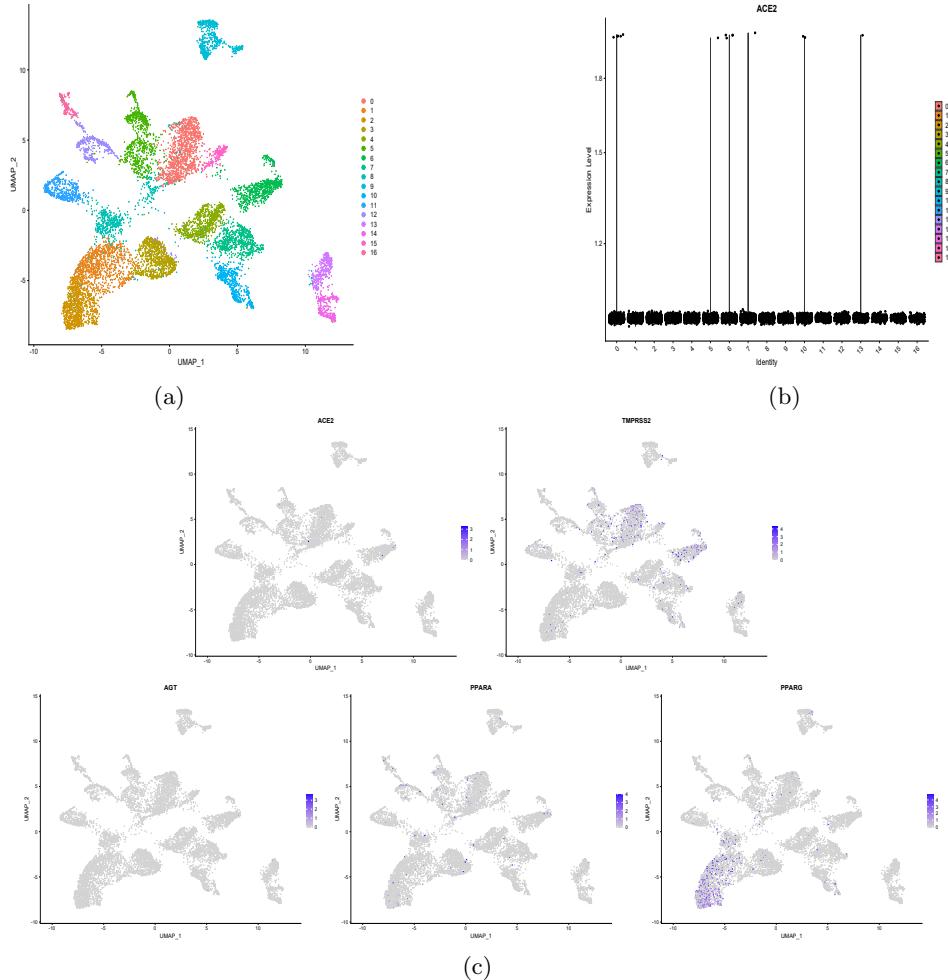
PAT2 are detected as potential cells in Lung where ACE2, AGT and PPARA show significant expression. In Fig. 1 the violin plot of the ACE2 expression level which implies that lung is highly vulnerable towards viremia. However, we have also identified two different cells i.e., plasma cell and Mast cell. In the case of plasma cells, all three transcripts are significantly expressed whereas in Mast cells AGT and ACE2 are significantly expressed. For the PAT2 cells and Mast cells, the functional hub is shown in Supplementary Fig. 1.

#### 3.2 Cell Specific Functional Hubs from Bladder and Kidney

From Bladder, ACE2 shows higher affinity in urothelial cells. The expression level is reported in Fig. 2 The functional Hub (shown in Supplementary Fig. 2) consists of 11 samples. Unlike, PPARG from PPAR family is showing significant expression level. From Kidney, ACE2 is showing higher affinity in Proximal Tubule Cells and Smooth Muscle Cells (shown in Fig 3. In Supplementary Fig. 3, the functional hub for Proximal Tubule Cells has shown. The involvement of the AGT, ACE2 and PPARA remain same in these two cell types. The rest of the samples are given in Table 1.

#### 3.3 Cell Specific Functional Hubs from Ileum and Liver

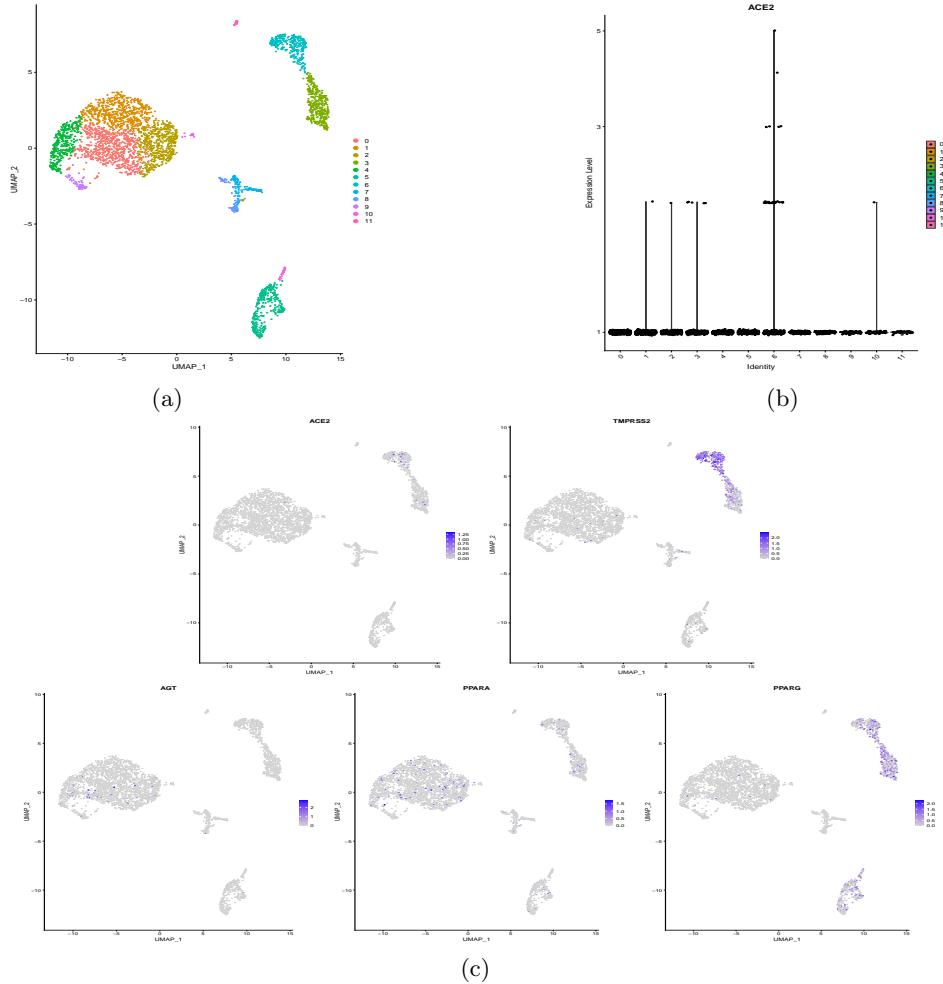
Ileum and Liver have taken as a part of the metabolic systems. Both of the organs have ACE2 positive epithelial-like cells. In the case of ileum, Enterocyte progenitor cells and Ciliated Epithelial cells have a significant expression for ACE2, AGT and PPARA (Fig 4). Fig. 5 reveals the expression level is low rather than other organs. The rest of the samples are given in Table 1. The functional hubs of the cells are shown in Supplementary Fig. 4 and Fig. 5. Similarly, cholangiocytes is one of the epithelium cell classes essentially found in liver tissue. The markers trio ACE2, AGT and PPARA are detected with higher expression value in these cell-types.



**Fig. 1.** Lung single cell RNA-seq data analysis showed that ACE2 is highly expressed in Pulmonary Alveolar type II cell. (a) The diverse cell-types present in bladder are categorized into 17 clusters. (b) Violin plot is used to show the expression level distribution of ACE2 across the clusters. (c) The feature expression of the potential hub genes identified from protein-protein interaction network with ACE2.

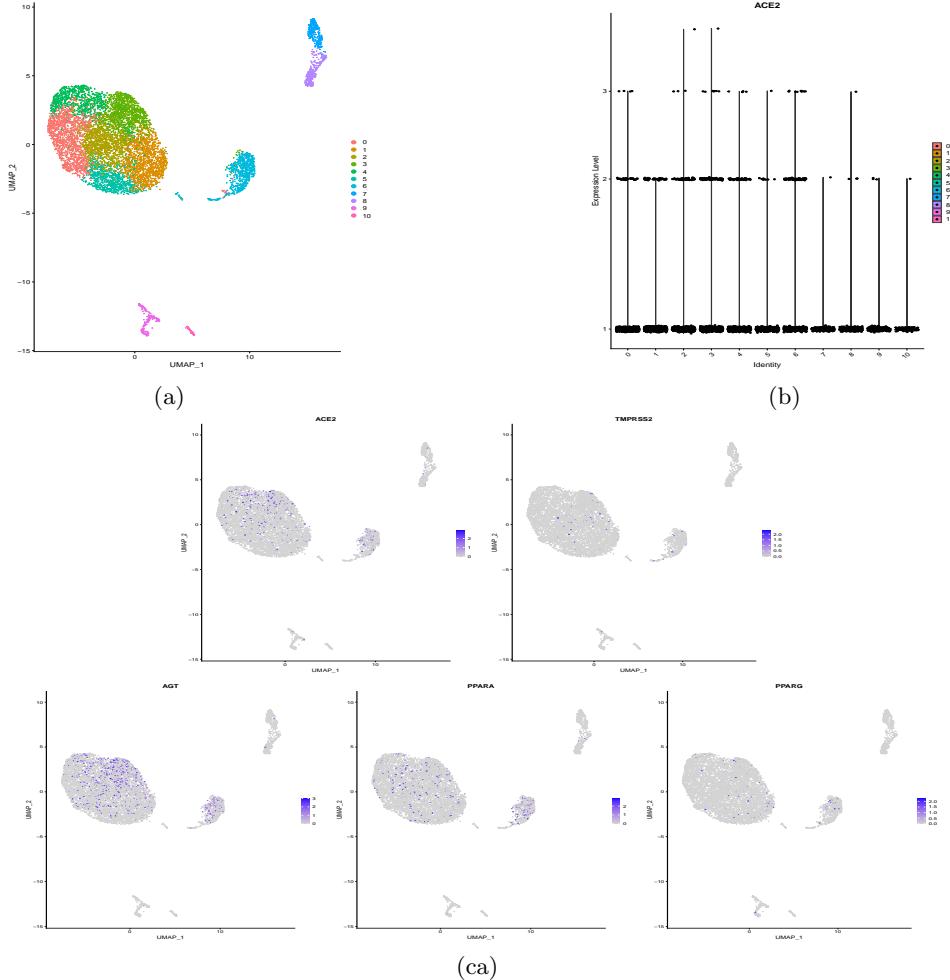
### 3.4 Enrichment Analysis

The genes corresponds to the potential hub from each PPIN <https://string-db.org/> of the cell-types those revealed high expression of ACE2 are considered to perform Gene Ontology (GO) analysis (Kuleshov et al., 2016). GO is the standardized annotation process to investigate the biological involvement of the gene product in a particular organism. In Table 2, we reported the biological process that shows maximum participation of the genes considered from PPIN. Inter-



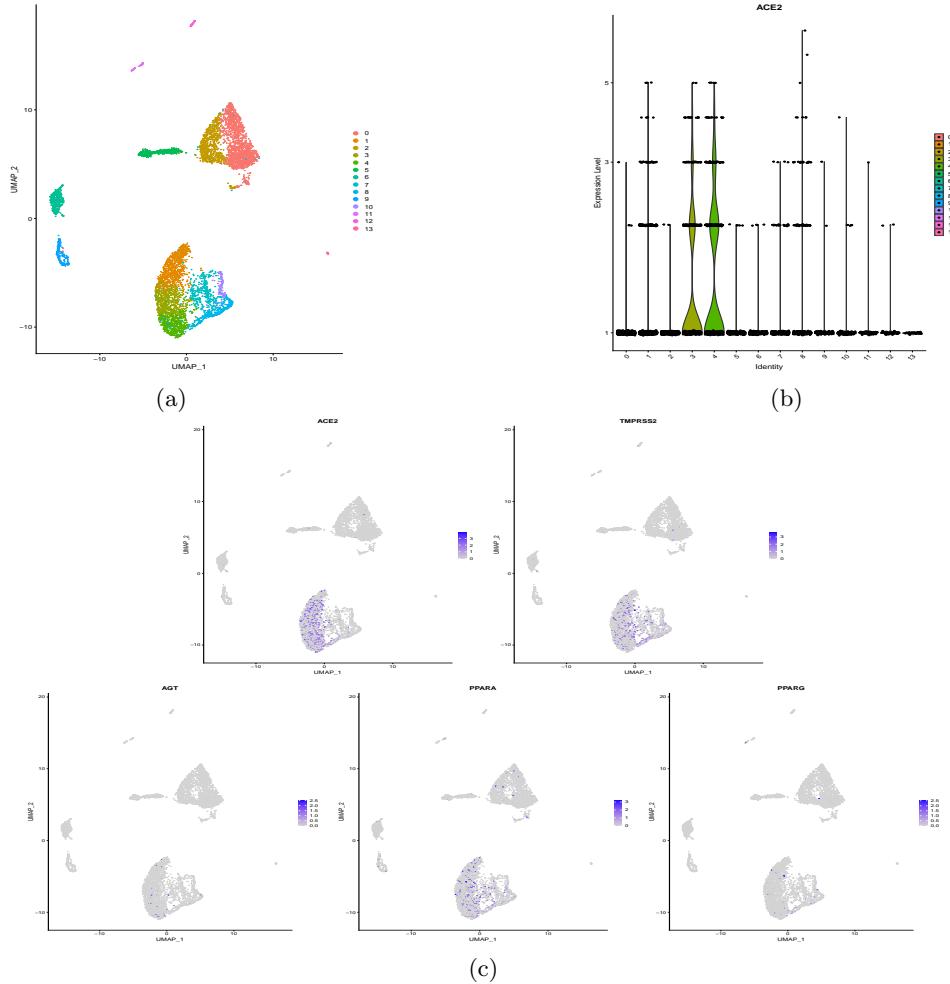
**Fig. 2.** Bladder single cell RNA-seq data analysis showed that ACE2 is highly expressed in Urethelial cell. (a) The diverse cell-types present in bladder are categorized into 12 clusters. (b) Violin plot is used to show the expression level distribution of ACE2 across the clusters. (c) The feature expression of the potential hub genes identified from protein-protein interaction network with ACE2.

estingly, we found some process that has direct or indirect collaboration with SARS-COV-2. During the study it is found that, biological processes such as Regulation of systemic arterial blood pressure by renin-angiotensin (Kreutz et al., 2020), Positive regulation of NF-kappaB transcription factor activity (DeDiego et al., 2014) and Regulation of cytokine production (Cao, 2020) is actively related to the target. This implies that not only ACE2 but the associated genes also play a key role during SARS-COV-2.



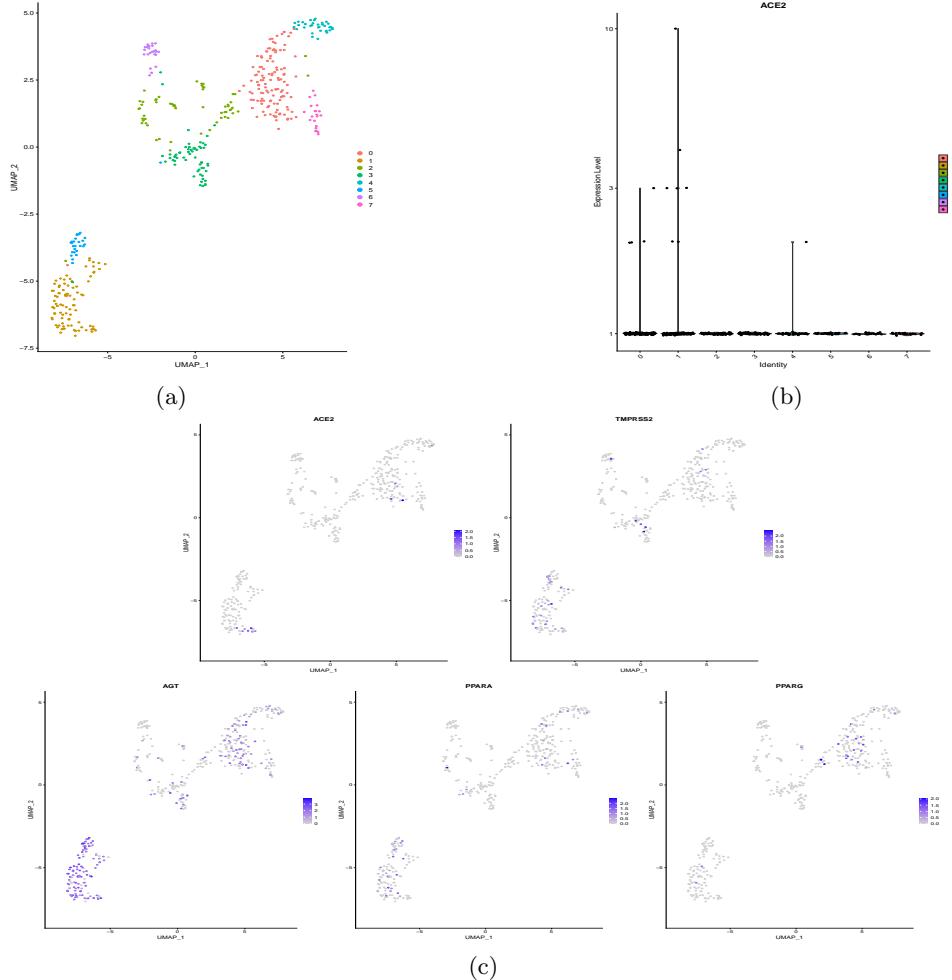
**Fig. 3.** Kidney ScRNASeq data analysis uncovered that Proximal tubule cells highly expressed ACE2. (a) The diverse cell-types present in bladder are categorized into 11 clusters. (b) Violin plot is used to show the expression level distribution of ACE2 across the clusters. (c) The feature expression of the potential hub genes identified from protein-protein interaction network with ACE2.

The cell-specific findings have provided a trio of a positive rate of significance viz., ACE2, PPAR family samples (in Bladder PPARG) and AGT. As per the potential cell types, if ACE2 and TMPRSS2 are co-expressed (Zhang et al., 2000), ACE2 has a higher propensity of associating with broader functional hubs. Co-expressed genes, AGT and PPAR/PPAG can act as connection between the functional hubs. PPAR family proteins are mostly tissue specific immune systems such as dendritic cells. A. Erol had reported the importance of



**Fig. 4.** The ScRNASeq data analysis of Ileum revealed that ACE2 is highly expressed in Enterocyte progenitor cell. (a) The diverse cell-types present in bladder are categorized into 14 clusters. (b) Violin plot is used to show the expression level distribution of ACE2 across the clusters. (c) The feature expression of the potential hub genes identified from protein-protein interaction network with ACE2.

PPARG in pioglitazone study on nCoV infection (Erol, 2020). Singla et al. have reported a therapeutic strategy statin for acute lung injury. The study has been introduced anti-inflammatory effects through peroxisome proliferator-activated receptor- $\gamma$  and transforming growth factor- $\beta$  (Singla & Jacobson, 2012). Therefore, the importance of the PPAR family proteins in drug reusability for acute lung diseases can be established. Similarly, many different articles have reported the importance of the peroxisome induced immune response against COVID-19



**Fig. 5.** The ScRNASeq data analysis of Ileum revealed that ACE2 is highly expressed in Cholangiocytess. (a) The diverse cell-types present in bladder are categorized into 8 clusters. (b) Violin plot is used to show the expression level distribution of ACE2 across the clusters. (c) The feature expression of the potential hub genes identified from protein-protein interaction network with ACE2.

infection (Taghizadeh-Hesary & Akbari, 2020; South, Diz, & Chappell, 2020). On other hands, AGT and ACE2 are directly involved with renin-angiotensin system (RAS) (South, Tomlinson, Edmonston, Hiremath, & Sparks, 2020). Huang et al. have reported the RAS inhibition for H7N9 infection (Huang et al., 2014). Also, Kuster et al. report the possibility of RAS inhibition as a therapeutic chance associated with COVID-19 (Kuster et al., 2020). AGT and PPAR family pro-

**Table 1.** The five human organ with their potential hub genes in highly expressed cell-types.

Human Organ	Cell-type	Significant genes
Bladder	Urethelial cell	FAM3B,STINK1,ACE2,AGT,PPARG,FABP4,FABP5,AQP3,SLC14A1
Ileum	Enterocyte	DPEP1,ACE2,AGT,PPARA,AEPEP,FABP1,PCK1,ALDOB,TPT1
Kidney	Proximal tubule cells	ACE2,AGT, SLC3A1,CLU,RBP4,FABP1,PPARA
	Principal cells	ACE2,NPC2,APOE,APOC3,CLU,ALB,IGFBP7
	Smooth muscle cells	ACE2,AGT,PPARA,MLXIPL,FAM118A,HSPA1B,JUN
Liver	Cholangiocytess	ACE2,AGT,CLU,SPP1
Lung	PAT2 cells	ACE2,AGT,PPARA,FABP5,NPC2
	Plasma cells	ACE2, AGT,PPARA,FOS,C5PR1,FPR1
	Mast cells	ACE2,AGT,CTSG,GPR65,ANXA1

**Table 2.** The biological processes associated with the significant genes in the important cell-types of the five organs.

Organ	Cell-type	Significant genes	Biological process	Adjusted P-value
Bladder	Urethelial	FABP4,FABP5	GO:0019433: Triglyceride catabolic process	0.065
		ACE2,PPARG,AGT	GO:0050727: Regulation of inflammatory response	0.054
		PPARG,AQP3	GO:0032526: Response to retinoic acid	0.120
Ileum	Enterocyte	ALDOB,PCK1	GO:0006090: Pyruvate metabolic process	0.055
		FABP1,DPEP1	GO:2000117: Negative regulation of cysteine-type endopeptidase activity	0.100
		FABP1,DPEP1,TPT1	GO:0043069: Negative regulation of programmed cell death	0.137
Kidney	PT cells	ACE2,AGT	GO:0003081: Regulation of systemic arterial blood pressure by renin-angiotensin	0.011
		ACE2,PPARA,AGT	GO:0031325: Positive regulation of cellular metabolic process	0.008
		FABP1,PPARA,AGT	GO:0080090: Regulation of primary metabolic process	0.009
Liver	Cholangiocytess	ACE2,AGT	GO:0002003: Angiotensin maturation	0.004
		CLU,AGT	GO:0051092: Positive regulation of NF-kappaB transcription factor activity	0.077
		SPP1,AGT	GO:1903508: Positive regulation of nucleic acid-templated transcription	0.262
Lung	PAT2	NPC2,PPARA,AGT	GO:0019216: Regulation of lipid metabolic process	0.002
		ACE2,AGT	GO:0001817: Regulation of cytokine production	0.085
		FABP5,NPC2	GO:0043312: Neutrophil degranulation	0.371

tein have a vital position in cellular functional hubs, associated with the ACE2. There is a therapeutic possibility associated with these two category samples.

## 4 Conclusion

The single cell-based bioinformatic strategy, applied in this article, has aimed to unveil the organ-specific probable infected cell types. One of the objectives of the study is to identify the possible functional hubs associating with ACE2 dysregulation during SARS-COV-2 infection. In the study, we have identified cell types from five different organs. Also the each of the potential cell types has a list biomarkers that are identified as interacting functional hubs based ACE2. Interestingly, AGT and PPAR family transcripts are common in each of the functional hubs. These two transcripts are acted as the connector between ACE2 and the rest of the samples from the functional communities. As per literature, angiotensin and PPAR family proteins are previously observed to participate in different COVID like infections. Also, the corresponding ontology terms of the markers are associated with the general cell health and primary immunity. Hence, it has been suspected that these samples can be influential druggable candidate.

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