Single-cell RNA expression profiling shows that ACE2, the putative receptor of Wuhan 2019-nCoV, has significant expression in the nasal, mouth, lung and colon tissues, and tends to be co-expressed with HLA-DRB1 in the four tissues

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Abstract

A novel coronavirus (2019-nCoV) was first identified in Wuhan, Hubei Province, and then spreads to the other Provinces of China. 2019-nCoV was reported to share the same receptor, Angiotensin-converting enzyme 2 (ACE2), with SARS-CoV. Previous studies have found ACE2 is abundantly present in humans in the epithelia of the lung and small intestines, and they found ACE2 expression in the basal layer of the non-keratinizing squamous epithelium in nasal and oral mucosa and the nasopharynx. Here based on the public single-cell RNA-Seq datasets, we analyzed the ACE2 expression in the nasal, mouth, lung, and colon tissues. We find that the number of ACE2-expressing cells in the nasal tissue and mouth is comparable to the number of ACE2-expressing cells in the lung tissue and colon. We also find that ACE2 tends to be co-expressed with HLA-DRB1, which plays a central role in the immune system by presenting peptides derived from extracellular proteins, in the nasal, mouth, lung, and colon tissues at single-cell resolution. We hope this provides valuable information for virus-prevention strategy and therapeutic strategy development.

Introduction

Severe infection by 2019-nCoV could result in acute respiratory distress syndrome (ARDS) and sepsis, causing death in approximately 2% of infected individuals[1]. Once contacted with the human airway, the spike proteins of 2019-nCoV can bind the surface receptors of

sensitive cells, and mediate the virus to enter into target cells. Recently, Xu et al. modeled the spike protein and indicated that Angiotensin-converting enzyme 2 (ACE2) could be the receptor for the 2019-nCoV[2]. Zhou et al. showed that ACE2 is essential for 2019-nCoV to enter HeLa cells[3]. These results indicated that ACE2 is likely to be the receptor for 2019-nCoV. Once the putative receptor of 2019-nCoV is identified, an urging work is to identify the potential host cells of 2019-nCoV in the human body comprehensively.

Previous studies have investigated the RNA expression of ACE2 in 72 human tissues[4]. They found ACE2 is abundantly present in humans in the epithelia of the lung and small intestines. They also found ACE2 expression in the basal layer of the non-keratinizing squamous epithelium in nasal and oral mucosa and the nasopharynx. However, the experiment is conducted at the bulk level. The number of cells expressing ACE2 in each tissue could not be estimated. Yu Zhao et al. analyzed the single-cell RNA-Seq data of the lung tissues from eight donors and found that ACE2 is mainly expressed in the type II alveolar cells (AT2)[5]. They also found that the number of ACE2-expressing AT2 in an Asian donor is much higher than the other seven non-Asian donors, which suggest the lung of the Asian could be a niche for 2019-nCoV replication.

HLA-DRB1 belongs to the HLA class II beta chain paralogs. It plays a central role in the immune system by presenting peptides derived from extracellular proteins. HLA-DRB1 is widely expressed in antigen-presenting cells (APC: B lymphocytes, dendritic cells, macrophages) and other cells. Within the DR molecule of HLA-DRB1, the beta chain contains all the polymorphisms specifying the peptide binding specificities. People have made intensive studies to explore the association between the polymorphisms of HLA-DRB1 and human diseases such as asthma, multiple sclerosis, and rheumatoid arthritis[6]. People also found that the polymorphisms of HLA-DRB1 are associated with our susceptibility to Human Immunodeficiency Virus (HIV)[7], herpes simplex virus(HSV)[8], BK virus[9], Hepatitis C Virus(HCV)[10], hepatitis B virus (HBV)[11], Epstein-Barr virus (EBV)[12], and Influenza[13]. The relationship between the polymorphisms of HLA-DRB1 and the susceptibility to Severe acute respiratory syndrome coronavirus (SARS-CoV) was also investigated, but no consistent conclusions were made[14,15].

In current work, we analyzed ACE2 single-cell expression profiles in the non-immune cells of the nasal, mouth, lung, and colon tissues. We find that 2.5% non-immune nasal tissue cells, 2% non-immune mouth tissue cells, 5.6% non-immune lung tissue cells, and 2.8% epithelial cells of the colon have ACE2 expression. For the non-immune tissue cells, the percent of ACE2-expressing cells in the nasal tissue and mouth is comparable to the percent of ACE2-expressing cells in the lung tissue and colon epithelial tissue. We also find that ACE2 tends to be co-expressed with HLA-DRB1 in the non-immune cells of the four tissues at single-cell resolution. We hope our work provides valuable information for virus-prevention strategy and therapeutic strategy development.

Result

2.5% non-immune nasal tissue cells, 2% non-immune mouth tissue cells, 5.6%

non-immune lung tissue cells, and 2.8% non-immune colon tissue cells express ACE2

Sandra Ruiz García et al. profiled single-cell gene expression of airway epithelium from nasal brushing of one donor, bronchial biopsy of a donor, and turbinate of a donor[16]. Reyfman, P. et al. sequenced the single-cell gene expression of the lung (bronchioli terminales) from eight donors[17]. The gene expression of bronchial epithelium from 12 donors was profiled by Duclos GE et al. at single-cell resolution[18]. Puram SV et al. sequenced squamous cell carcinoma tissue of mouth from 18 patients at single-cell resolution[19]. Smillie CS et al. profiled single-cell gene expression of colon epithelial cells from 12 healthy individuals[20].

Using Seurat, we performed unsupervised graph-based clustering on these single-cell RNA-Seq datasets. Then, We ran the Uniform Manifold Approximation and Projection (UMAP) dimensional reduction technique to visualize the data. Next, we used the violin plot to find the clusters with significant PTPRC (CD45) expression in the datasets and filtered out the clusters. Thus, we got non-immune cells in the datasets. At last, we used the violin plot to visualize ACE2-expressing cells in each cluster in each dataset.

At first, we find that ACE2 is not abundantly expressed in any cluster in the datasets (Figure 1). It suggests that 2019-nCoV may not infect a significant number of cells of a cell-type. The damage of 2019-nCoV to a non-immune cell-type maybe limit. Then we calculated the percent of ACE2-expressing cells in each dataset (Table 1). We find that, in nasal tissue, 2.5% non-immune cells from nasal brushing and 1.7% non-immune cells from turbinate have ACE2 expression. 2% non-immune malignant and normal cells from mouth show ACE2 transcription. In lung tissue, 0.2% non-immune cells from the bronchial biopsy, 5.6% non-immune cells from bronchial brushings, and 1.1% non-immune cells from bronchioli terminales have ACE2 transcription. 2.8% non-immune cells from colon epithelial cells express ACE2. For the mouth, bronchial brushing, lung (bronchioli terminals), and colon tissues, multiple donors/patients were sequenced. We calculated the percent of ACE2-expressing cells in non-immune cells per donor/patient (Table 1). We find that the percent of ACE2-expressing cells in non-immune cells calculated by the collected samples is rather reliable and not dominated by an outlier donor/patient with a very high percent of ACE2-expressing cells in non-immune cells.

ACE2 tends to be co-expressed with HLA-DRB1 in the nasal, mouth, lung, and colon tissues at single-cell resolution

The violin plots of the distribution of ACE2 and HLA-DRB1 in the clusters of the non-immune cells of the mouth, turbinate, nasal brushing, bronchial biopsy, bronchial brushing, lung, and colon tissues suggest that the two genes appear to be co-expressed (Figure 1). We employed the hypergeometric test to test the co-appearance of ACE2 expression and HLA-DRB1 transcription in the non-immune cells. We find that ACE2 is co-expressed with HLA-DRB1 in all the datasets, excluding the dataset of bronchial biopsy, at single-cell resolution (Table 1; P-value <0.05).

In the single-cell RNA-Seq datasets of the mouth, bronchial brushing, lung (bronchioli terminals), and colon tissues, multiple donors/patients were sequenced. We analyzed the co-expression of ACE2 and HLA-DRB1 in the non-immune cells of individual donor/patient. We find that, in four of the eight patients of mouth tissue, ACE2 is co-expressed with

HLA-DRB1 (Table 1; P-value <0.05). We noticed that in the four patients in whom ACE2 failed to be significantly co-expressed with HLA-DRB1, the number of total sequenced cells and ACE2-expressing cells is limit (Table 2). In contrast, the percents of ACE2-expressing cells in the HLA-DRB1-expressing cells are higher than the percent of HLA-DRB1-expressing cells in total cells, which suggests the enrichment of ACE2-expressing cells in the HLA-DRB1-expressing cells (Table 2). In the four patients, we believe that ACE2 will be significantly co-expressed with HLA-DRB1 if more cells are sequenced.

In only one of the eight donors of bronchial brushing tissues, ACE2 is co-expressed with HLA-DRB1 (Table 1; P-value <0.05). However, the number of total sequenced cells and ACE2-expressing cells is limit in the other donors who have in-significant P-value. The percents of ACE2-expressing cells in the HLA-DRB1-expressing cells are higher than the percent of HLA-DRB1-expressing cells in total cells (Table 2). We infer that ACE2 should be significantly co-expressed with HLA-DRB1 if more cells are sequenced.

In four of the eight donors of lung tissues, ACE2 is co-expressed with HLA-DRB1 (Table 1; P-value <0.05). We find that, in the four patients in whom ACE2 failed to be significantly co-expressed with HLA-DRB1, the percents of ACE2-expressing cells in the HLA-DRB1-expressing cells are higher than the percent of HLA-DRB1-expressing cells in total cells (Table 2).

In eight of the twelve donors of colon epithelial tissue, ACE2 is co-expressed with HLA-DRB1 (Table 1; P-value <0.05). We find that, in three of the four patients in whom ACE2 failed to be significantly co-expressed with HLA-DRB1, the percents of ACE2-expressing cells in the HLA-DRB1-expressing cells are higher than the percent of HLA-DRB1-expressing cells in total cells (Table 2).

Given the above, we conclude that ACE2 tends to be co-expressed with HLA-DRB1 in the nasal, mouth, lung, and colon tissues

Discussion

Previous studies have found ACE2 is abundantly present in humans in the epithelia of the lung and small intestines and also expressed in nasal and oral mucosa and the nasopharynx[4]. With single-cell RNA-Seq technology, we precisely calculated the number of ACE2-expressing cells in nasal, mouth, lung, and colon tissues and find the number of ACE2-expressing cells in nasal and mouth tissues is comparable to the number of ACE2-expressing cells in the lung and intestine tissues. We think we should make more effort to figure out whether the nasal and mouth epithelial cells are the first major host cells of 2019-nCoV-infection, which may help us understand why so many people are infected with 2019-nCoV but have few or no clinical symptoms.

HLA-DRB1 plays a central role in the immune system by presenting peptides derived from extracellular proteins. The polymorphisms of HLA-DRB1 have been demonstrated to be associated with our susceptibility to diseases and viruses-infection. We have found that ACE2 is co-expressed with HLA-DRB1 in the tissues with abundant 2019-nCoV host cells. Thus, one urging work is to investigate whether 2019-nCoV-infection would significantly elevate HLA-DRB1 expression in the lung.

We admit the single-cell profiling of mouth tissue with squamous cell carcinoma is not an ideal model to study ACE2 expression in mouth tissue. However, it is the only single-cell

RNA-Seq data that we can find having profiled mouth tissue at single-cell resolution. The malignant cells are derived from normal epithelial cells of the mouth; we believe most of the malignant cells should still maintain their tissue specificity.

Method

The single-cell RNA-Seq datasets of mouth, bronchial brushing, lung (bronchioli terminales), and colon epithelial tissues were downloaded from GSE103322, GSE131391, GSE122960, and SCP259 (Single Cell Portal). The single-cell RNA-Seq datasets of turbinate, nasal brushing and bronchial biopsy were downloaded from GSE121600.

Single-cell RNA-Seq dataset pre-processing

We employed Seurat (3.1.4) to process the single-cell RNA-Seq datasets. At first, we filtered out the cells 1-expressing less than 200 genes; or 2-highly expressing mitochondrial genes, in which mitochondrial genes' reads account for more than 25% of the total reads. We filtered out the genes expressing in less than 3 samples. Then, we got the processed single-cell RNA-Seq datasets.

Single-cell RNA-Seq dataset clustering and visualization

We employed Seurat in default mode to cluster and visualize cell-clusters (See supplemental file S1.txt for the R code).

Identification of the non-immune cells

We used the violin plot to check the PTPRC expression in each cluster in each single-cell RNA-Seq dataset. The cluster whose PTPRC expression having a spindle body in the violin plot was filtered. Thus, we got the non-immune cells of each single-cell RNA-Seq dataset.

Test the significance of enrichment of ACE2-expressing cells in HLA-DRB1-expressing cells

We employed the hypergeometric test to test the significance of enrichment of ACE2-expressing cells in HLA-DRB1-expressing cells. Supposed N is the number of total sequenced cells, M is the number of HLA-DRB1-expressing cells, K is the number of ACE2-expressing cells, we calculated the possibility (p) of finding x or more than x cells of ACE2 expression and HLA-DRB1 expression when we randomly picked K cells from total sequenced cells (N). We used R function phyper to calculate p as follow,

$$\mathrm{p} = 1 - \mathrm{phyper}((\mathrm{x}-1), \mathrm{M}, (\mathrm{N}-\mathrm{M}), \mathrm{K});$$

where $\mathrm{K} > 3$, we do not calculate p for the data whose $\mathrm{K} \leq 3$.

Acknowledgments

This article is dedicated to the people who are fighting with 2019nCoV

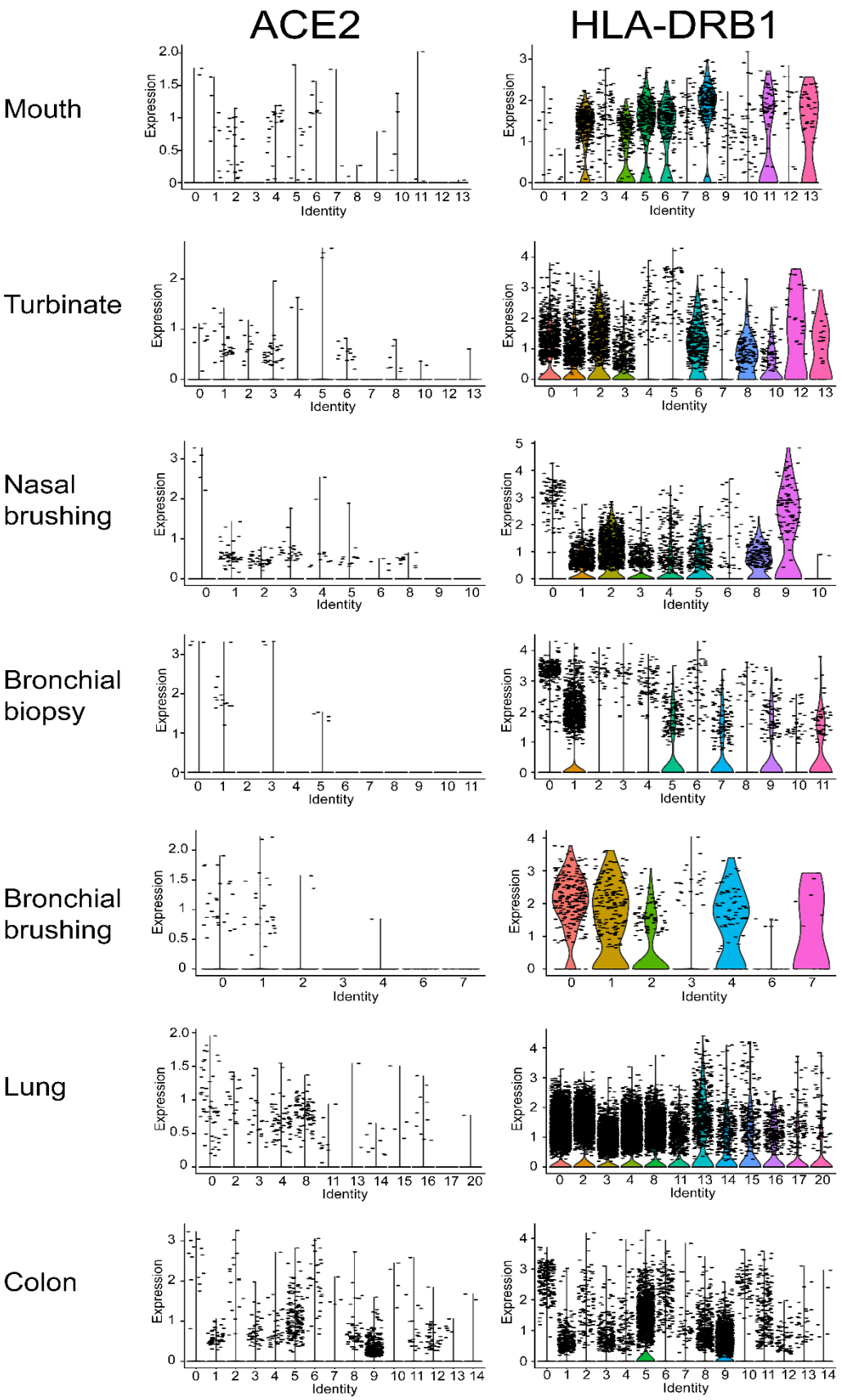

Figure 1: The violin plot of ACE2 and HLA-DRB1 in the clusters of non-immune cells from nasal, mouth, lung, and colon tissues

Table 1: Number of total sequenced cells, ACE2-expressing cells, HLA-DRB1-expressing cells and cells with both HLA-DRB1 expression and ACE2 expression in the nasal, mouth, lung and colon tissues

Samples	Total Cells	ACE2	ACE2/Total cells	HLA-DRB1	HLA-DRB1&ACE2	P-value
Mouth 17 samples collected	4239	85	2.0%	1511	66	1.6E-15
Sample1-HNSCC7	7	1	14.3%	1	0	-
Sample2-HNSCC8	62	0	0.0%	37	0	-
Sample3-HNSCC9 or 23	53	0	0.0%	15	0	-
Sample4-HNSCC10	37	0	0.0%	6	0	-
Sample5-HNSCC12	16	1	6.3%	3	1	-
Sample6-HNSCC13	31	1	3.2%	22	1	-
Sample7-HNSCC16	256	1	0.4%	71	1	-
Sample8-HNSCC17	413	21	5.1%	204	18	4.7E-04
Sample9-HNSCC18	331	6	1.8%	209	6	6.2E-02
Sample10-HNSCC20	691	8	1.2%	13	6	3.2E-10
Sample11-HNSCC22	178	4	2.2%	119	4	2.0E-01
Sample12-HNSCC24	108	1	0.9%	11	1	-
Sample13-HNSCC25	495	2	0.4%	78	1	-
Sample14-HNSCC26	415	11	2.7%	215	9	4.0E-02
Sample15-HNSCC28	575	18	3.1%	182	17	2.4E-08
Sample16-HNSCC5	274	4	1.5%	203	4	3.0E-01
Sample17-HNSCC6	297	6	2.0%	122	4	1.9E-01
Turbinate	6066	106	1.7%	2827	74	9.3E-07
Nasal brushing	6424	163	2.5%	2507	108	1.0E-12
Bronchial biopsy	10795	24	0.2%	1695	6	1.6E-01
Bronchial brushings 12 samples collected	921	52	5.6%	547	46	2.5E-06
Sample1-Never smoke 1	81	6	7.4%	64	5	6.3E-01
Sample2-Never smoke 2	82	5	6.1%	39	5	2.1E-02
Sample3-Never smoke 3	84	4	4.8%	51	4	1.3E-01
Sample4-Never smoke 4	82	6	7.3%	67	5	7.0E-01
Sample5-Never smoke 5	75	4	5.3%	31	3	7.0E-01
Sample6-Never smoke 6	85	1	1.2%	50	1	-
Sample7-Current smoke 1	70	2	2.9%	27	1	-
Sample8-Current smoke 2	87	4	4.6%	66	4	3.2E-01
Sample9-Current smoke 3	31	1	3.2%	9	0	-
Sample10-Current smoke 4	86	13	15.1%	73	13	9.9E-02
Sample11-Current smoke 5	73	2	2.7%	18	1	-
Sample12-Current smoke 6	85	4	4.7%	52	4	1.3E-01
Lung 8 samples collected	25291	280	1.1%	14862	220	1.5E-12
Sample1-Donor1	2591	21	0.8%	2004	17	4.7E-01
Sample2-Donor2	2526	94	3.7%	1845	81	1.5E-03

Sample3-Donor3	2381	14	0.6%	1356	13	4.3E-03
Sample4-Donor4	3623	11	0.3%	2099	8	2.5E-01
Sample5-Donor5	3352	25	0.7%	2724	23	1.3E-01
Sample6-Donor6	2175	36	1.7%	1342	29	1.2E-02
Sample7-Donor7	5045	25	0.5%	1978	14	6.6E-02
Sample8-Donor8	3598	54	1.5%	1514	35	5.8E-04
Colon 12 samples collected	30038	828	2.8%	4088	466	0.0E+00
Sample1-N10	4175	46	1.1%	54	2	1.2E-01
Sample2-N11	3412	32	0.9%	493	15	1.1E-05
Sample3-N13	515	7	1.4%	32	2	6.5E-02
Sample4-N15	4619	124	2.7%	702	62	0.0E+00
Sample5-N16	1539	31	2.0%	23	1	3.8E-01
Sample6-N17	1409	30	2.1%	476	22	9.0E-06
Sample7-N18	2288	51	2.2%	52	7	1.1E-04
Sample8-N20	1193	9	0.0%	4	0	1
Sample9-N21	2371	93	3.9%	662	51	2.3E-08
Sample10-N46	1850	60	3.2%	103	14	2.3E-06
Sample11-N51	6024	329	5.5%	1395	282	0.0E+00
Sample12-N8	643	16	2.5%	92	8	6.4E-04

We assume the number of total single cells, ACE2-expressing cells, HLA-DRB1-expressing cells, and cells with both HLA-DRB1 expression and ACE2 expression follows hypergeometric distribution. We calculated the P-value of the hypergeometric test to measure the significance of enrichment of ACE2-expressing cells in HLA-DRB1-expressing cells. However, we omitted the data whose ACE2-expressing cells is less than 4.

Table 2: The percent of HLA-DRB1-expressing cells in total single cells and the percent of ACE2-expressing cells having HLA-DRB1 expression in the individual donor/patient whose P-value >=0.05

Tissue	Donor/patient	Total Cells	ACE2	HLA-DRB1/Total cells	ACE2&HLA-DRB1/ACE2	P-value
Mouth	Sample9-HNSCC18	331	6	0.63141994	1	6.20E-02
	Sample11-HNSCC22	178	4	0.668539326	1	2.00E-01
	Sample16-HNSCC5	274	4	0.740875912	1	3.00E-01
	Sample17-HNSCC6	297	6	0.410774411	0.666666667	1.90E-01
Bronchial brushings	Sample1-Never smoke 1	81	6	0.790123457	0.833333333	6.30E-01
	Sample3-Never smoke 3	84	4	0.607142857	1	1.30E-01
	Sample4-Never smoke 4	82	6	0.817073171	0.833333333	7.00E-01
	Sample5-Never smoke 5	75	4	0.413333333	0.75	7.00E-01
	Sample8-Current smoke 2	87	4	0.75862069	1	3.20E-01
	Sample10-Current smoke 4	86	13	0.848837209	1	9.90E-02
	Sample12-Current smoke 6	85	4	0.611764706	1	1.30E-01
Lung	Sample1-Donor1	2591	21	0.773446546	0.80952381	4.70E-01
	Sample4-Donor4	3623	11	0.579354126	0.727272727	2.50E-01
	Sample5-Donor5	3352	25	0.812649165	0.92	1.30E-01
	Sample7-Donor7	5045	25	0.392071358	0.56	6.60E-02
Colon	Sample1-N10	4175	46	0.012934132	0.043478261	1.20E-01
	Sample3-N13	515	7	0.062135922	0.285714286	6.50E-02
	Sample5-N16	1539	31	0.014944769	0.032258065	3.80E-01
	Sample8-N20	1193	9	0.003352892	0	1

P-value of hypergeometric test is calculated to measure the significance of enrichment of ACE2-expressing cells in HLA-DRB1-expressing cells.

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