

The molecular basis of loss of smell in 2019-nCoV infected individuals

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Short Report

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Abstract

Among the prominent clinical symptoms such as fatigue, shortness of breath, fever, and cough, 2019-nCoV infected individuals often experience hyposmia/anosmia (decrease or loss of sense of smell). Angiotensin I Converting Enzyme 2 (ACE2), a key host receptor has now been established as an important moiety for the entry of 2019-nCoV into the host cells. A multitude of studies estimated the expression of *ACE2* in multiple organs including heart, kidney, intestines, lungs, buccal cavity, etc. The ongoing medical examinations and the autopsy reports of the diseased individuals strongly corroborate these organ/tissue-level molecular insights. Olfactory mucosa harbors multiple functionally distinct cell types. Zeroing in on the cell lineages that underpin infection associated loss of olfaction may provide new leads for diagnostics/clinical management of 2019-nCoV infected individuals. Our pointed bioinformatic analysis of single-cell expression profiles underscored selective expression of *ACE2* in a subset of horizontal basal cells (HBCs) and sustentacular cells (SUSs) of the olfactory mucosa in humans. Inspection of the *ACE2* levels in the olfactory mucosa of 4 additional mammalian species revealed comparable expression patterns, indicating the risk of olfactory dysfunction in these species. In summary, our findings pinpoint the molecular rationale of loss of smell in 2019-nCoV infected patients.

Introduction

The recent outbreak of the novel coronavirus 2019-nCoV triggered the urgent requirement of diagnostic methods, which can be rapidly deployed and therefore, timely employed by masses across the world [1–4]. In pursuit of this, various workgroups have extensively generated, curated and analyzed virus-centric datasets [5,6]. Some major efforts include virus isolation from the airway epithelial cells and its genome sequencing [5–7]. Comparative genomics revealed that 2019-nCoV is closely related to bat SARS-like coronaviruses (bat-SL-CoVZC45 and bat-SLCoVZXC21) [5]. Notably, the external subdomain of Spike's Receptor-Binding Domain (RBD) of 2019-nCoV shares ~40% identity at the amino acid level with other SARS-related coronaviruses [8]. Of note, most of the amino acid differences of the RBD are located in the external subdomain, which is responsible for the direct interaction with the host receptors. Further, reports indicate the role of angiotensin-converting enzyme II (ACE2) as a prominent surface receptor for the cellular entry of 2019-nCoV [9,10]. Mechanistic insights further revealed the involvement of viral S-protein in assisting strong interaction with the host ACE2 receptor [11]. All these studies collectively reinforce the involvement of ACE2 in the viral entry into the host cell. In order to determine the tissue or organ level impact of 2019-nCoV, various groups have traced *ACE2* expression in multiple organs/cell-types [12–15]. Notably, many of these studies have leveraged single-cell sequencing technology to pin-point the cell subpopulation of interest. Collectively, higher *ACE2* expression was observed in a range of tissue/cell-types such as epithelial cells of the esophagus, absorptive enterocytes of the intestines, mucosal cells of the oral cavity, proximal tubule cells of the kidney, myocardial cells of the heart, urothelial cells of the bladder, etc, thereby making them potentially vulnerable to the 2019-nCoV infection [12–15]. All these molecular findings are largely in line with the clinical symptoms reported worldwide, in which multi-organ failure is emerging as a major contributor to the infection associated mortality

We leveraged some recently published high-throughput single-cell expression studies to re-evaluate *ACE2* expression levels among cell types of the olfactory mucosa. Our analysis revealed selective expression *ACE2* in a subset of HBCs and SUSs. Notably, marginal expression was observed in a subset of Bowman's gland and globular basal cells, whereas no expression was observed in other cell types such as olfactory ensheathing glia, microvillar cells, immature or mature olfactory sensory neurons.

Results

Predominant and selective expression of *ACE2* in a subpopulation of olfactory-specific horizontal basal cells and sustentacular cells

We evaluated the expression of *ACE2* transcript in 3906 olfactory mucosa originated single cells from the recent report by Durante and colleagues [16], collectively entailing eight distinct olfactory cell types namely horizontal basal cells, microvillar cells, Bowman's gland cells, globular basal cells, olfactory ensheathing glia, sustentacular cells, immature and mature olfactory sensory neurons. We performed unsupervised clustering of the individual cells using the Seurat software suite [17]. Clusters were unambiguously mapped to specific cell types based on previously known markers (Fig. 1A). Our analysis revealed that the expression of *ACE2* is restricted to a subset of sustentacular and horizontal basal cells of the olfactory mucosa, collectively comprising less than 1% of the total cells (Fig. 1B). Notably, a minor sub-fraction of the globular basal cells and Bowman's gland cells also exhibited *ACE2* expression (Fig. 1C). To determine if the *ACE2*-positive (*ACE2*⁺) subpopulation of HBCs and SUSs are indeed functionally distinct from the *ACE*-negative (*ACE2*⁻) cells, we performed differential expression analysis between two subgroups of the horizontal basal cells (HBC⁺; *ACE2*⁺ and HBC⁺; *ACE2*⁻), segregated based on *ACE2* expression. Gene ontologies referring to the biological processes revealed significant distinction among the subpopulations of horizontal basal cells (Fig. 1D). Similar results were obtained for sustentacular cells (SUS⁺; *ACE2*⁺ and SUS⁺; *ACE2*⁻) (Fig. 1E). In both cases, the functional distinction of the subpopulation was found to be linked to the key molecular processes related to cellular homeostasis and cell cycle (Fig. 1D-E).

Comparable *ACE2* expression in the olfactory mucosa in mammals

Recent studies indicate that the high rate of transmission 2019-CoV2 is remarkably higher as compared to the related SARs-CoV. It is now being proposed that such a high transmission rate is attributed to genetic recombination events at the S protein of the RBD region [18]. Although it has been speculated that the 2019-CoV is transmitted to humans from animal sources [18], little is known about the capability of the other mammal species to act as carriers. Notably, in a recent report, monkeys have been confirmed to have the ability to be carriers of this infection [19]. We asked if other common mammalian species are also at the risk of developing 2019-CoV mediated loss-of-olfaction. To test this, we estimated the levels of *ACE2* transcripts in single-cell transcriptomes, sampled from the olfactory mucosa of 5 mammalian species. Our results indicate that the relative abundance of *ACE2* in the olfactory mucosa is comparable in humans and monkeys, whereas significantly higher levels were observed in marmoset, mouse, and rat

(Fig. 1F). These analyses clearly suggest that similar to humans, the olfactory system of other mammals is also at potential risk of 2019-CoV infection.

Discussion And Future Directions

In addition to the infection-induced multi-organ dysfunction, a major bottleneck in combating the pandemic outbreak of 2019-CoV is the availability and accessibility of the diagnostic methods to masses worldwide. Although major improvements have been made in developing 2019-nCoV centric molecular diagnostic kits, their timely availability to the masses across worldwide will require a substantial amount of time and resources. Recently, multiple clinicians indicate the abrupt loss of smell in a large number of 2019-nCoV infected individuals, particularly from Britain, the US, France, South Korea, China, Germany, and Iran, therefore, collectively reinforce its potential application as the first line of diagnostics in the patients possessing 2019-CoV hallmark symptoms. Although these symptoms are prevalent in the majority of 2019-CoV infected individuals, so far the impacted cell type of the olfactory mucosa mediating these phenotypes is still unknown. We report here that loss of smell in the infected patients is most unlikely due to the direct impairment of the olfactory sensory neurons. Moreover, our results indicate that the sustentacular cells and the horizontal basal cells are the potential cell types that are highly susceptible to viral entry (Fig. 1G). Although, the supporting cells (SUS) and horizontal basal cells (HBCs) are not sensory in function, but are known to play a crucial role in the maintenance of the olfactory organ [20–23]. Although little is known about the exact role of sustentacular cells in influencing the sensing functionality of the receptor neurons, however, they are known to provide metabolic and physical support to the olfactory mucosa. Similarly, is the case with HBCs, where their direct or indirect involvement in influencing odorant detection is not reported, however, they are known to play a crucial role in the regeneration of the olfactory epithelium upon lesions [24,25]. Due to the potential damage of the reserve olfactory stem cells and the crucial supporting cells upon 2019-nCoV infection, it is most likely that even after recovery, the patients may encounter loss of smell until the cellular composition of the olfactory mucosa reestablishes. Collectively, our study provides the first line of evidence that a subpopulation of olfactory cells is potentially equipped with cell surface receptors which can be exploited by the virus for gaining entry into the cell.

Methods

For the considered single-cell studies, raw read count data were downloaded from GEO (GSE139522). We performed the majority of our analyses including cell/gene filtering, clustering and differential expression analysis using the widely used Seurat software suite [17]. Inbuilt functions `NormalizeData()`, `FindVariableFeatures()`, `ScaleData()`, `RunPCA()`, `DimPlot()`, `FindNeighbors()` and `FindClusters()` were used for the various standard steps of single-cell expression data analysis. Notably, annotation of the cell clusters was performed using the set of markers indicated in the original study [16]. R script used for the analysis can be found at shorturl.at/PT245.

Uniformly processed bulk RNA sequencing data possessing transcriptomic profiles of whole olfactory mucosa from 5 mammalian species i.e. human, monkey, marmoset, mouse, and rat were downloaded from a recent publication from Saraiva and colleagues [26]. FPKM values were log-transformed (base=2) and used for plotting the bar charts. The student's t-test was used to calculate the differences in the mean values between all species. A p-value < 0.05, <0.01 and < 0.001 is denoted as (*), (**), and (***), respectively.

Declarations

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Competing interests

The authors declare that they have no competing interests.

Contributions

The study was conceived by GA and DS. Experimental workflows were designed by GA, DS, TM, and performed by KG, SKM, SK, AM. Clinical insights were provided by JA. Illustrations were drafted by GA and AM. GA and DS wrote the paper. All authors have read and approved the manuscript.

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Figures

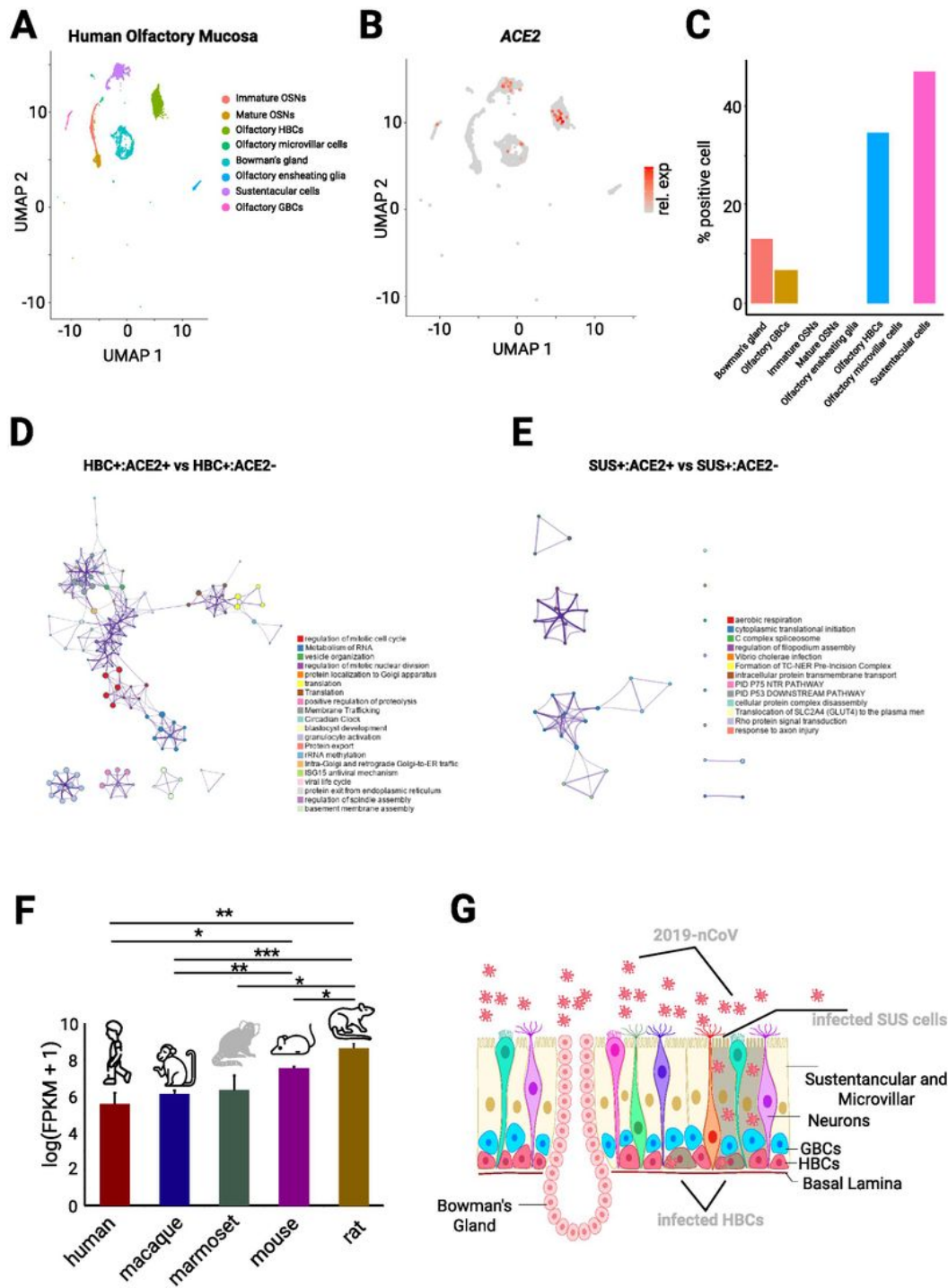


Figure 1

Specialized expression of ACE2 in a subpopulation of sustentacular and horizontal basal cells of olfactory mucosa (A) UMAP based embedding of single-cell expression profiles represents the distinct cell types of the olfactory mucosa, segregated-based on their transcriptional profiles. Cells within a cluster represent similar cell types. (B) UMAP based embedding portrays the relative expression of ACE2 transcript in the indicated olfactory mucosa cell types. (C) Percentage bar graphs representing the relative proportion of ACE2 expressing cells in different cell types. (D-E) Metascape analysis of the differentially expressed genes between ACE2-positive and ACE2-negative cells revealing the prominent functional ontologies segregating SUSs and HBCs cellular populations. (F) Bar graph depicting the relative abundance of ACE2 transcript in the olfactory mucosa of 5 indicated mammalian species. (G) Graphical representation of the key findings.