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Spotlight

Can't smell the virus: SARS-CoV-2, chromatin organization, and anosmia

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Anosmia, or loss of smell, is strongly associated with SARS-CoV-2 infection in humans, but the underlying mechanism remains obscure. In a recent *Cell* study, Zazhytska et al. (2022) report non-cell-autonomous disruption of long-range genomic interactions of olfactory receptor genes in response to SARS-CoV-2 infection, and these interactions remain disrupted long after virus clearance.

Transient anosmia, an inability to smell, is a common symptom associated with many upper respiratory viral infections, including SARS-CoV-2. Typically, upon infection, nasal congestion blocks the odorants in sensory neurons, causing loss of smell. Anosmia in SARS-CoV-2 infection, however, occurs independently from nasal symptoms, and it may last long after the infection is resolved. In addition, the SARS-CoV-2 host cell entry proteins are not expressed by olfactory sensory neurons (OSNs) (Bilinska et al., 2020), and this makes viral infection of these neurons exceedingly rare. These pathological and molecular features raise the possibility that non-cell-autonomous mechanisms may underlie the anosmia that is frequently induced by SARS-CoV-2 infection.

In a recent study published in *Cell*, Zazhytska et al. (2022) explored the non-cell-autonomous molecular changes that occur in response to SARS-CoV-2 infection. As a model, they used golden hamsters (*M. auratus*), which have a host cell receptor, ACE2, through which SARS-CoV-2 enters cells (Bilinska et al., 2020) and which is highly similar to the human ortholog. They therefore closely resemble humans in SARS-CoV-2-related pathogenicity and immunogenicity (Cleary et al., 2020; Sia et al., 2020). To explore the changes in cellular composition and gene expression following SARS-CoV-2 infection, the authors collected olfactory epithelium (OE) from sham-infected or SARS-CoV-2-infected golden hamsters 1, 3, and 10 days post-infection, and performed single-cell RNA-sequencing (scRNA-seq). They identified 13 different cell types and found, as expected, that sustentacular (SUS) cells, which express the ACE2 receptor, comprise the large majority of the infected cells. Although microglia and assorted immune cells also demonstrated substantial viral uptake, only a small fraction of the OSNs were infected. The cellular effects of direct viral infection were evinced by the fraction of SUS cells decreasing substantially (from ~20% to ~6%), whereas viability of OSNs remained unaffected. By day 10, the original cellular composition was restored, and the virus was no longer detected in any of the OE cells.

Zazhytska et al. (2022) then analyzed transcriptional changes in OE cells and, remarkably, found a significant reduction in genes related to olfaction, including transcription factors which regulate olfactory receptors (OR) and OR signaling genes. A notable example is adenylyl cyclase 3 (*Adcy3*), whose loss leads to anosmia in mice (Wong et al., 2000). But why are OR genes dysregulated following SARS-CoV-2 infection? During OSN maturation, the lamin B receptor protein (*LBR*) is downregulated, and suppressive heterochromatin is rearranged to form nuclear hubs, thus generating "fried-egg"-like inverted nuclei which contain both heterochromatin cores and multi-enhancer hubs (Pourmorady and Lomvardas, 2022). Active OR alleles are arranged in proximity to their corresponding enhancers to enable active mono-allelic transcription. Using Hi-C experiments in sham- and SARS-CoV-2-infected hamsters, the authors found disruption of long-range genomic interactions of OR genes happening already 1 day post-infection (Figure 1). By day 3, they detected more widespread and global chromatin rearrangements. Remarkably, OR gene rearrangements persisted at day 10 post-infection, when the virus is already completely cleared, and this potentially explains the persistent changes in expression of OR-related genes and the long-lasting anosmia that is associated with SARS-CoV-2 infection.

The low susceptibility of OSNs to viral entry, together with evident OR chromatin and transcriptional changes, suggests that anosmia is not the result of a direct viral mechanism. To test whether these effects were indeed non-cell-autonomous, the authors performed an elegant experiment. They collected serum from SARS-CoV-2-infected hamsters, whereupon UV irradiation was used to inactivate the virus prior to administration of the irradiated serum directly into the nasal cavity of virus-naïve hamsters. The irradiated serum not only caused a comparable disruption in nuclear architecture to that of SARS-CoV-2-infected hamsters, but, when examined merely 12.5 h after the nasal administration, when the effect of the released cytokines is at its peak, it resulted in faster kinetics than SARS-CoV-2 infection. Subtle reduction in OR gene expression was already detectable at this early timepoint. Taken together, these experiments demonstrate global changes in nuclear architecture related to OR genes in OSNs, caused by components, cellular or viral, that are released from other infected cells,

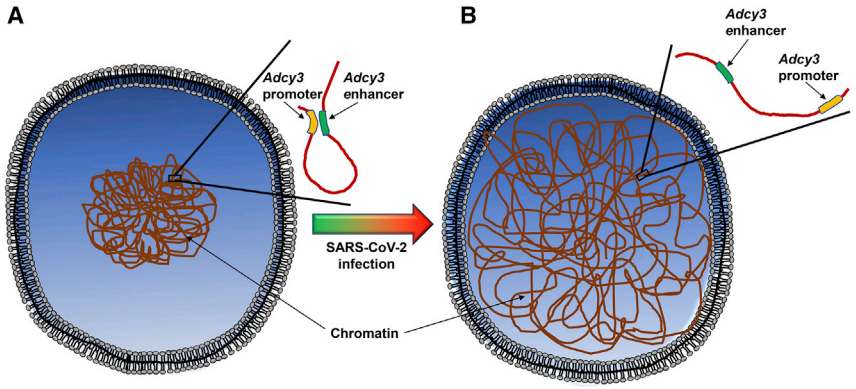

Figure 1. SARS-CoV-2 infection leads to non-cell-autonomous genome rearrangement in olfactory sensory neurons

(A) Olfactory sensory neurons (OSNs) have a typical "fried-egg"-like nuclear conformation in which large chromatin domains are clustered around the center of the nucleus. This unique chromatin organization ensures both silencing via juxtaposition to heterochromatin and activation through mediating long-range enhancer-promoter interactions, e.g. of the *Adcy3* gene (inset).

(B) Upon SARS-CoV-2 infection of neighboring sustentacular (SUS) cells, global rearrangement of chromatin in OSNs leads to disruption of long-range interactions between enhancers (green) and promoters (yellow) and to the down-regulation of OR-related genes.

presumably the infected SUS cells, and that operate in *trans*.

The authors' next goal was to assess the relevance of these findings in humans. Working with postmortem human tissues, the authors compared changes in gene expression between SARS-CoV-2-infected individuals and controls. They found a selective reduction in the expression of OR-related genes in human autopsies, including *Adcy3* and other key olfactory transcripts. Furthermore, comparing Hi-C interaction maps between OSNs from SARS-CoV-2-infected human autopsy specimens and OSNs from control autopsies, the authors found large-scale loss of long-distance interactions, many of which were specific to OR-related genes.

Overall, these results support the model that non-autonomous disruption of long-range genomic interactions of OR-related genes underlies SARS-CoV-2-induced loss of smell. Such disruptions in nuclear architecture may not be readily re-established in post-mitotic cells such as OSNs, and thus they may also explain the persistence of the anosmia phenotypes for weeks and months after infection, as well as other related phenotypes such as parosmia, a distortion in the sense of smell.

This study raises several interesting questions. Although it is clear from the experiments that the mechanism for the loss of smell is non-cell-autonomous and does not require live virus, the specific molecules that are responsible are yet to be identified. Possibilities include non-viable viral particles, molecules secreted from infected cells such as cytokines, and circulating fragments of SUS cells, as dying cells are constantly being shed into the bloodstream. It is also unclear how such a molecule would elicit the profound changes in nuclear architecture in OSNs.

Transcriptional studies of SARS-CoV-2 have revealed that the expression of many epigenetic factors correlate with an interferon response and with general activation of transposable elements (Sorek et al., 2022). Therefore, if, as the authors hypothesize, cytokines are responsible for the non-cell-autonomous anosmia, interferon activation in the OSNs may cause changes in expression of these factors, which can lead to the observed chromatin-related phenotype. Another outstanding question is, how specific is the response? Can other neurons or non-dividing cells in general be similarly affected? Can other viruses elicit similar pathologies? Are cells with unique nuclear architectures more susceptible than other cells? Such emerging questions will no doubt be the focus of follow-up investigations.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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