SINGLE-CELL SECRETS

Research on single cells revealed new insights into SARS-CoV-2, the opioid crisis, neurogenesis, and fertility.

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NEW EVIDENCE FOR NEURON PRODUCTION IN ADULT HUMANS

A single-cell RNA sequencing approach uncovered neural stem and progenitor cell populations in the olfactory bulb.

By Nathan Ni, PhD

In the absence of direct evidence to the contrary, many scientists concluded that neurogenesis in humans stopped after childhood or by the age of 13. However, a study recently published in *Nature Neuroscience* reopened the debate. Using a single-cell RNA sequencing (scRNA-seq) approach, Bradley Goldstein from Duke University and his research team found neural stem cell and progenitor cell populations in the adult olfactory bulb, providing direct evidence of neurogenesis in adults and suggesting that neurogenesis may continue for decades after childhood.

Previous studies disclosed neurogenic niches in various animal models, including rats and non-human primates. However, researchers found it difficult to relate animals, with their dramatically shorter life spans, to human adulthood, which potentially spans decades. Goldstein's team explored humans directly by obtaining fresh tissue samples from adults undergoing endoscopic nasal surgery and examining them at the genetic and protein levels.

Goldstein's group analyzed more than 28,000 individual cells using scRNA-seq. The technique enabled them to distinguish between cells from olfactory and respiratory epithelial regions, thus allowing the researchers to focus specifically on the olfactory region. When analyzing the data, Goldstein's team first recognized that the G protein subunit guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-8 (GNG8) distinguished immature neurons from mature counterparts, which express GNG13. Based on this stratification criteria, immature neurons formed the majority (55%) of all human olfactory neurons present.

The researchers also found and characterized a cluster of neurogenic globose basal cells (GBCs) during their investigation of olfactory cells. This population comprised approximately 2% of all olfactory neuroepithelial cells and was defined by expressing basic helix-loop-helix transcription factors such as transcription cofactor HES-6 (HES6), neurogenin-1 (NEUROG1), or neurogenic differentiation factor 1 (NEUROD1). Notably, the team observed clear differences in gene expression between GBCs, immature neurons, and mature neurons, depicting the step-wise transitions from progenitor to mature cells.

Finally, Goldstein's team examined olfactory receptor expression in order to characterize neuronal functionality. Across all neurons analyzed, they found 545 different olfactory receptors stemming from 140 different olfactory receptor genes. While only 50% of all immature neurons expressed at least one olfactory receptor, that proportion increased to >85% for the mature neuron population. The study also confirmed the "one-neuron/one-receptor" rule, with 75% of mature olfactory sensory neurons (OSNs) expressing only one olfactory receptor.

In this study, Goldstein's group provided direct evidence for continuing robust neurogenesis in the adult human olfactory neuroepithelium. Using scRNA-seq, they characterized individual cell populations representing stages along the journey from stem cell to mature neuron. This information may pave the way for neuroregeneration research.

Goldstein's group provided direct evidence for continuing robust neurogenesis in the adult human olfactory neuroepithelium.

Reference

M.A. Durante et al., "Single-cell analysis of olfactory neurogenesis and differentiation in adult humans," *Nat Neurosci*, 23:323-26, 2020.

OPIOIDS SUPPRESS ANTIVIRAL GENES IN MULTIPLE IMMUNE CELL TYPES

Researchers use single-cell RNA-seq to investigate the influence of chronic and short-term opioid use on the immune system.

By Niki Spahich, PhD

Opioid usage has skyrocketed in the past twenty years, and has become the main cause of drug overdose deaths in the United States. Researchers agree that prolonged opioid usage, either prescription or illicit, negatively impacts the central nervous system, causing symptoms ranging from sleep disturbances to cognitive impairment. Opioid drugs also impair both the innate and adaptive immune systems through poorly understood mechanisms, and opioid users are more susceptible to opportunistic infections, such as HIV, tuberculosis, and pneumonia. Because opioid drug usage is so prevalent, it is important to understand its holistic effects on the body.

Recently, a team led by Christine Cheng from Boston University explored how opioid drugs affect the immune system. Previous studies on these drugs and the immune response have been inconsistent, with some claiming that opioids are immunosuppressive while others suggest that they are immunoactivating. These studies often focused on a few genes and single immune cell subpopulations. Cheng's team set themselves apart by taking a genome-wide approach and analyzing the transcriptomes of primary immune cells from chronic opioid users using single-cell RNA sequencing (scRNA-seq).

The researchers performed microdroplet-based scRNA-seq on peripheral blood mononuclear cells (PBMCs) from seven opioid-dependent individuals and seven control individuals. They found that chronic opioid usage suppressed viral defense and interferon signaling genes in naïve monocytes.

Cheng's team took a genome-wide approach and analyzed the transcriptomes of primary immune cells from chronic opioid users.

Next, the researchers analyzed the effect of lipopolysaccharide (LPS), a component of the Gram-negative bacterial outer membrane, on PBMCs from chronic opioid users by scRNA-seq to determine the immune response after pathogen stimulation. LPS activates several innate immune response pathways, including the antiviral response. After a 3-hour in vitro treatment, the scientists observed the repression of antiviral and interferon signaling genes in a variety of innate and adaptive immune cell subtypes, including CD4+ T cells, CD8+ T cells, activated T cells, B cells, and NK cells. Treating PBMCs from healthy individuals with morphine prior to LPS exposure resulted in a similar suppression of antiviral genes in several immune cell subpopulations. These data suggest that both chronic and acute opioid exposure can negatively alter the immune response.

LPS stimulates the antiviral response in several ways, including through the TLR4 receptor pathway, by inactivating the TRIF signaling cascade, which leads to gene activation by type I interferons. To determine if the gene suppression in cells from chronic opioid users depended on this pathway specifically, the researchers directly stimulated the antiviral gene program using interferon beta. In this case, they found no difference between control and test cell populations, which suggests that opioid-induced gene suppression indeed depends on the TLR4 pathway.

Chronic opioid users are susceptible to infection for several reasons. Prescription users are often battling cancer using immunosuppressive treatments or are post-surgery, and many recreational users use needles to inject the drugs. These data suggest an additional reason for their susceptibility and highlight the need for careful monitoring of each opioid user to avoid comorbidity with infectious diseases.

Reference

T.T. Karagiannis et al., "Single cell transcriptomics reveals opioid usage evokes widespread suppression of antiviral gene program," *Nat Comm*, 11:2611, 2020.

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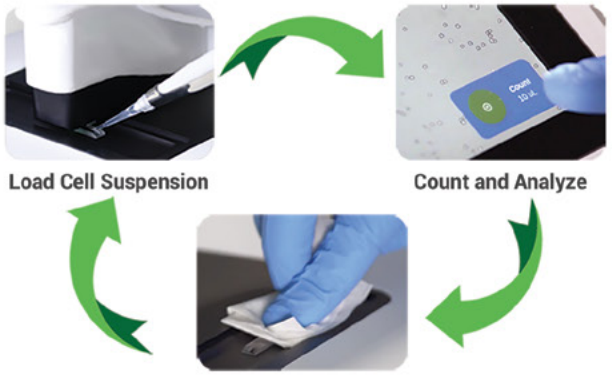

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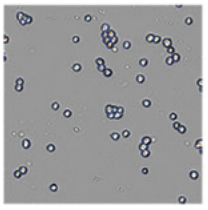

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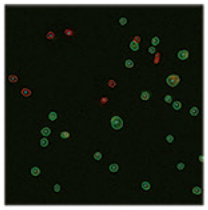

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SARS-COV-2 AFFECTS SOME LUNGS MORE THAN OTHERS

Two single-cell techniques show that ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells.

By Kathryn Loydall, PhD

In late 2019, the novel coronavirus SARS-CoV-2 jumped into humans in the Hubei Province of China and rapidly began spreading disease around the world. Many researchers immediately began learning as much as possible about the virus to help combat the pandemic. Lung cancer researchers at the German Center for Lung Research (DZL) were among those who altered their research focus; using existing yet unpublished data from single-cell techniques to investigate viral receptor and cofactor gene transcripts, the team found that the virus affects certain lung cells more than others.

Roland Eils, founding director of the Berlin Institute of Health (BIH) Digital Health Center, and his colleagues from the Thorax Clinic at Heidelberg Hospital initially intended to study lung cancer in people who have never smoked. In preparation for this study, before the COVID-19 pandemic, they acquired and analyzed healthy and diseased tissue samples from twelve lung cancer patients from the Heidelberg Lung Biobank. They also obtained and analyzed cells from the airways of four healthy patients collected during non-invasive bronchoscopy examinations.

When the COVID-19 pandemic took hold, however, the researchers switched gears. The scientists knew from previous studies that the coronavirus used its surface spike (S) glycoprotein to engage an angiotensin-converting enzyme 2 (ACE2) receptor on the cell surface, and that the virus needed one or more cofactors to penetrate cells. The team wondered which specific cells the coronavirus infiltrated. To find out, they re-examined the single-cell data from the uninfected patients to determine which lung cells contained the gene for ACE2 and the cofactor transmembrane protease serine 2 (TMPRSS2), which primes the viral S protein to allow cell entry.

Working cell-by-cell, Eils' team analyzed single-nuclei and single-cell RNA sequencing data for *ACE2* and *TMPRSS2* expression from nearly 60,000 cells from lung tissue and bronchial branches. The researchers found the gene transcripts for ACE2 and TMPRSS2 in small numbers. They saw *TMPRSS2* expression in both tissues, but *ACE2* was expressed predominantly in progenitor cells in the bronchial branches—cells that normally develop into respiratory tract cells lined with cilia that sweep mucus and bacteria out of the lungs. Interestingly, these transiently differentiating cells showed an enrichment for pathways related to Rho GTPase function and viral processes suggesting increased vulnerability for SARS-CoV-2 infection.

Eils' team also noted that the ACE2 receptor density on the cells increased with age and was generally higher in men than in women, mirroring the patterns of disease severity seen in older male patients.

Working cell-by-cell, Eils' team analyzed single-nuclei and single-cell RNA sequencing data for ACE2 and TMPRSS2 expression from nearly 60,000 cells.

These data suggest that the virus acts in a highly selective manner, and that it depends on certain human cells to spread and replicate. To find out if this is the case, the researchers next plan to use single-cell techniques to investigate bronchial transient secretory cells and immune cells from COVID-19-infected patients. They want to better understand why the infection takes a benign course in some patients, while causing severe disease in others.

Reference

S. Lukassen et al., "SARS -CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells," *EMBO J*, 39(10):e105114, 2020.

UNRAVELING INDIVIDUAL SPERM DNA

A new technique for single-cell sperm sequencing reveals insights into sperm meiosis.

By Tiffany Garbutt, PhD

Aneuploidy, an abnormal number of chromosomes in germ cells, can lead to infertility, miscarriage, or conditions such as Down syndrome. Researchers have thoroughly characterized aneuploidy in egg cells, where it arises from defects in meiosis. Meiosis in sperm cells, however, is a mystery.

Studies of sperm focus mainly on cell count and motility, largely because sperm genomes are tightly wound and compacted, making them less accessible to researchers. Combined with the fragility of sperm, this makes it particularly difficult for researchers to evaluate sperm meiosis at the single-cell level. In a recent study published in *Nature*, researchers developed a novel method called Sperm-Seq that sequences the DNA of thousands of individual sperm cells in parallel and allows researchers to peer into the genomes of sperm and evaluate key meiotic events for the first time.

A team of researchers from Harvard Medical School and the Broad Institute of Massachusetts Institute of Technology and Harvard, led by Avery Davis Bell and Steven McCarroll collected sperm from 20 human donors ages 18 to 38 years. To make sperm DNA accessible, they used regents that mimicked the ability of egg enzymes to decondense the sperm pronucleus during fertilization. This caused the sperm DNA to emerge as a floret, which the researchers captured in individual droplets containing a barcoded bead. This approach combined three common single-cell sequencing technologies: Drop-seq, Chromium Single-Cell DNA, and GemCode. The team also developed new computational tools to evaluate chromosomal phase, ploidy, and meiotic crossover events.

Using their new approach, Sperm-Seq, the researchers captured and sequenced 31,228 individual sperm cells with a median 1% genome coverage for each cell. They inferred the parental haplotypes for each donor and identified crossover events based on switches in parental haplotype. Overall, they observed variation in meiotic events among donors, chromosomes, and cells.

Across all cells, the researchers observed 787 whole-chromosome aneuploids and 133 chromosome arm-level gains or losses. The average number of aneuploid sperm varied from person-to-person, with an average of 2.5%, a comparable amount to previous estimates made from microscopy assessments of subsets of chromosomes. On average, the researchers found that sex chromosomes were 2.2 times more likely to be affected by errors during meiosis I and that autosomes were twice as likely to be affected by errors in meiosis II.

The researchers evaluated a total of 813,122 crossover events and observed an average recombination rate of 22.2 – 28.1 crossover events per cell, which was also consistent with previous estimates. At large, most crossover events occurred at the distal ends of chromosomes. Some crossover events occurred near the centromere or middle of the chromosome, but this varied from donor to donor. Donors with higher rates of recombination were more likely to have crossover events near the centromere. Donors with higher rates of global crossover events also were more likely to have more crossovers on average on each chromosome.

The researchers noted that variation in the location and rate of crossover events, along with other meiotic phenotypes, may be due to underlying heritable factors. They also speculated that the degree of sperm genome compaction may also influence the location and number of crossover events and may in itself be heritable. In an effort to advance single-cell technology and aid genetic research, the authors made their Sperm-Seq protocols freely available.

Reference

A.D. Bell et al., "Insights into variation in meiosis from 31,228 human sperm genomes," *Nature*, 583:259-264, 2020.

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