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Editorial: Transforming toxicology one cell at a time: A special issue on the application of scRNA-seq to the study of environmental response

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Edited by **Kristie Willet**, **Patrick Allard** and **Justin Colacino**

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Introduction

Since the first report of single-cell RNA sequencing in 2009, scRNA-seq and related single-cell 'omics have become omnipresent in biological research. The analysis of gene expression at individual cell levels has transformed our understanding of cellular differentiation, carcinogenesis, development, aging, and many other fields by providing an unprecedented unbiased and comprehensive look into cells' molecular programs.

As with other fields of research, toxicology has also tremendously benefited from scRNA-seq. Specifically, this approach allows the profiling of cells to: (1) detect the earliest toxicity-associated molecular changes that may be hidden when performing bulk RNA-seq; (2) reveal changes in cell-type composition over time, for example during development, during disease progression, or aging; and (3) relatedly, investigate, in each tissue or organ examined, which cell types are most sensitive (as defined by the amplitude of transcriptional shift) to the chemical exposure.

A brief history of single-cell seq

The emergence of scRNA-seq can be tied to the development and scaling up of next-generation sequencing methods in the early 2000s. With increased throughput and decreasing sequencing costs, sequencing of RNA, albeit in bulk form, became standard for studying gene expression, providing average expression profiles of entire cell populations.

Some of the earliest examinations of individual cells' gene expression involved the use of glass pipettes to separate and isolate cells. The material was then processed towards the generation of cDNA libraries that served as a template for RT-PCR [1] or micro-array analysis [2–5]. This approach proved particularly valuable for delineating the distinct transcriptional program underlying the differentiation program of individual neurons [2,3]. The first use of mRNA sequencing of an individual cell was reported soon after, in 2009, with the manual isolation of mouse embryonic blastomeres and oocytes followed by single-cell cDNA preparation and sequencing [6].

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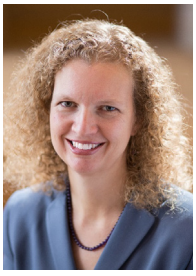

Kristine L. Willett, PhD is Chair of the Department of BioMolecular Sciences in the School of Pharmacy at the University of Mississippi. A professor of Pharmacology and Environmental Toxicology, she earned her BA in Chemistry at the University of North Carolina and PhD in Toxicology from Texas A&M University. She and her trainees study the developmental, reproductive, and multigenerational adverse outcomes of cannabinoids and benzo[a]pyrene exposure primarily using fish models.

A major breakthrough in the development of scRNA-seq came in 2015 through two concurrent technologies, Drop-seq and InDrops, in which microfluidics was combined with mRNA sequencing to generate encapsulated cells [7,8]. The barcoding of droplet-containing cells allowed the massive parallel single-cell sequencing with relative ease. The commercialization of this approach by 10x Genomics and their Chromium Single Cell 3′ platform dramatically increased access to scRNA-seq. Over the years, advances in scRNA-seq have continued to be made with improvements in library preparation, droplet-based systems, and, significantly, with the development of a large number of computational tools and pipelines for data analysis. ScRNA-seq has also been increasingly combined with other 'omics approaches, notably ATAC-seq, in order to refine cell clustering and deepen our biological understanding at the single-cell level. Recently, single-cell approaches have been adapted in the tissue context to allow for high-resolution spatial mapping of molecular signatures in a tissue using imaging and sequencing-based approaches.

From single cells to whole organisms

One significant advantage of scRNA-seq is its application at scale, from tissue to organs, and to whole organisms. This versatility of the approach lies in the identification of the appropriate cellular dissociation method, usually enzymatic or mechanical, that will favor cell isolation without compromising cellular integrity. Thus, starting from 2019, the report of the profiling of entire organisms, such as *Hydra* and *Caenorhabditis elegans* emerged [9–11]. These whole organs and organisms approaches opened the door to mapping cellular changes across development [9–11] or across the life course, i.e. during aging [12,13].

When cellular integrity is difficult to maintain or in tissues that are syncytial, a compromise can be found in the application of single-nucleus RNA-seq. The scalability of sc/sn-RNAseq represents a fantastic opportunity for toxicologists to combine the precision of a cell-level resolution with the comprehensiveness of a whole organ or whole organism approach [14].

Single-cell approaches and toxicology

The uptake of scRNA-seq by the field of Toxicology is recent and apparent starting in 2019/2020. Following the 2019 publication by Zhang and colleagues highlighting the value of applying scRNA-seq to toxicological questions [15], the following year's annual meeting of the Society of Toxicology saw the presentation of the first session dedicated to single-cell approaches entitled: "Single Cell Technologies: A Potentially Transformative Tool for Toxicology". Moving from its potentiality to its proven benefit, a 2022 SOT annual meeting session that also focused on scRNA-seq was entitled: "Applications of Single Cell Profiling Methods to Enhance Mechanistic Understanding of Toxicological Responses". Furthermore, the opening plenary session of the 2023 meeting, delivered by Dr. Namandjé Bumpus from the U.S. FDA was entitled: "Advancing Single Cell Technologies in Toxicology" which presented the clear advantage of single-cell proteomics to understand cell-to-cell variability in chemotherapeutic drug response in human [16]. The widespread adoption of scRNA-seq in Toxicology is also evidenced by the large number of presentations and abstracts at the most recent 2024 SOT annual meeting making use of scRNA-seq.

Summary of issue and goals of issue

The goal of this issue of COTOX was to cover how single-cell profiling is informing toxicological research questions in the context of different organ systems and disease models. It highlights the power of the approach to understand precise cell-specific mechanism-based responses across the dose and time course of toxic insult. In addition, perspectives are provided on the methodological considerations and limitations of the assays and on how these approaches can inform regulation.

Focusing on the liver, Dr. Rance Nault highlights on single-cell transcriptomics and has provided detailed insights into the distinct roles of liver cell types in 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)-induced liver damage and how TCDD disrupts the cellular microenvironment and architecture of the liver [17]. By leveraging these approaches, researchers have identified common and novel mechanisms in models of toxicant-associated fatty liver disease (TAFLD) and other fatty liver disease (FLD). Additionally, Jigang Wang and co-authors provide further perspectives of how single-cell approaches can inform mechanisms and cell type susceptibility of hepato- and nephrotoxicity following glyphosate and aristolochic acid exposures, respectively [18].

Dr. Alessandro Venosa presents compelling studies that highlight the significant gaps in our understanding of lung injury stemming from ozone exposure [19]. By applying single-cell sequencing to the study of ozone injury and combining this approach with computational inferences of cell-cell communication, single-cell platforms can reveal the cellular interplay that drives lung pathology following ozone exposure.

With regards to neurotoxicology, Tukker and Bowman cover several recent studies that have revealed the intricate transcriptional responses in the central nervous system to exposures such as lead, bisphenol AP, or triphenyl phosphate in rodent models [20]. The article also highlights how the combination of organoid-based approaches and single-cell transcriptomics can accelerate mechanistic discoveries in neurotoxicology. Sampson, Morgan et al. provide insights into the rigorous use of single-cell methods to understand the effects of exposure to the heavy metal lead throughout the life course, from neurodevelopment to neurodegeneration [21]. They highlight the importance of evaluating cellular heterogeneity in complex tissues, like the brain, and provide recommendations for data generation and analysis for future single-cell neurotoxicology studies.

Like neurotoxicity, immunotoxicity is also highly dependent on cell type heterogeneity. In her submission, Dr. Britton Goodale uses an arsenic case study to highlight the strengths of scRNA -seq to identify cell-type-specific responses not noted in tissue-level analyses and to identify pathology-relevant cell populations that may not be represented in *in vitro* model systems [22]. Future opportunities in biomarker identification and retrospective sequencing data analysis are suggested as ways to better understand the mechanisms of immunotoxicity. Dr. Peer Karmaus provides insights into immunotoxicology and metabolism, describing metabolic measures and the advantages of various approaches for quantifying metabolic outcomes at the single-cell level, ranging from FACS to emerging single-cell metabolomic methods [23]. Importantly, computational approaches for the quantification of metabolic states at the single-cell level are discussed, including a number of important case studies about the importance of understanding cell type heterogeneity in the content of immunometabolism and immunotoxicology.

By using current models of carcinogenesis such as the Hallmarks of Cancer and the Key Characteristics of Carcinogens frameworks, Aguilar and Colacino describe the use of both scRNA-seq and spatial transcriptomics in deciphering the cell-specific pathways involved in the development of breast cancer in response to carcinogen exposure [24].

Two articles consider how single-cell techniques can be leveraged to better understand reproductive and multigenerational toxicities. Using the zebrafish model organism, Dr. Tracie Baker and colleagues describe how reproductive tissue atlassing is being done to characterize gene expression in minor cell populations during development and reproductive organs [25]. Perchlorate and TCDD are provided as example toxicants that cause germ cell-specific gene expression changes revealed by single-cell transcriptomics. Dr. Patrick Allard and colleagues provide further examples of how single-cell approaches inform alcohol and e-cigarette reproductive toxicity using the *C. elegans* and mouse models, respectively [26]. They highlight how the highly complex and interactive aspects of reproduction and development are especially well-suited for single-cell and single-nuclei experimental designs so that the complex molecular networks can be understood.

Technical challenges

The rapidly evolving field of single-cell 'omics necessitates the development of systems biology approaches to interpret these complex data. Diamante, Ha et al. present a comprehensive overview of existing single-cell technologies and describe strategies for the analysis and integration of these data in a systems biology framework, with case studies highlighted from the toxicology literature [27].

As the field of single-cell RNA-seq is being applied more frequently to toxicological questions, the need to identify and establish best practices for analysis becomes increasingly apparent. Filipovic, Kana et al. tackle this challenge, providing essential guidance for key steps in the data analysis pipeline: data preprocessing, cell type identification, data integration and batch correction, clustering, differential cell abundance analysis, differential gene expression analysis, and quantification and comparison of cellular trajectories [28].

Future directions

The field of single-cell 'omics in toxicology is a rapidly growing field. For example, the development of multiomic and spatially informed single-cell measures have the opportunity to provide deep mechanistic insights into how chemical and biological agents impact cells and tissues. However, there is still a need to ensure that these methods are scalable to assess effects of agents in the traditional toxicological framework — considering the impacts across species, developmental stages, and sexes. Moreover, this is a continuing need for advancements in data analysis methods tailored for toxicological single-cell analyses. The articles in this issue provide a framework to apply single-cell approaches in toxicology from study conceptualization, to data generation, to analysis. We anticipate that single-cell approaches will have significant impacts across the domains of the field, from mechanistic toxicology, environmental/ecotoxicology, drug discovery, and risk assessment.

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