Improved Sequencing Method Leads to Advancements in Toxicology Research

NIEHS-funded Superfund Research Program scientists are employing a new RNA sequencing method to assess mechanisms of toxicity on a finer and more accessible scale. Researchers in SRP grantee Tim Zacharewski's Lab at the Michigan State University SRP Center conducted the study.

Using single-nuclei RNA sequencing, lead researcher Rance Nault and the team were able to identify distinct hepatic cell types, cell population shifts and relative abundances, and discriminated cell-specific pathways disrupted by 2,3,7,8-tetrachlorodibenzo-p-dioxin, or TCDD.

TCDD is an environmental contaminant produced from waste incineration, metal production, and fossil-fuel and wood combustion. TCDD is a potential contributing factor to complex metabolic diseases such as obesity, type II diabetes, and nonalcoholic fatty liver disease.

With the conventional method of bulk RNA sequencing, researchers would grind up whole pieces of tissue and measure the average levels of RNA for each gene in the sample. This method can mask the diversity of cell types in the tissue and misses how each type of cell responds to chemicals or drugs. The single-nuclei RNA sequencing method used in this study offers more information at a higher resolution about how TCDD affects each individual cell type.

Using frozen mouse liver samples treated with TCDD, the team compared single-nuclei RNA sequencing data to historical bulk RNA sequencing datasets. The comparison showed that the single-nuclei sequencing technology can reproduce previous findings, in addition to providing cell-specific information. The researchers captured major shifts in cell populations from these gene expression data and produced cluster graphs to visualize the cell-specific expression.

Single-cell RNA sequencing is the most common sequencing approach and can only be performed on freshly collected tissues. In typical toxicology study designs, multiple doses of chemicals, drugs, or supplements are used to establish their safety. The number of samples, coupled with the severe effects observed at higher doses, presents a significant hurdle in the use of freshly isolated single cells.

The MSU researchers were able to adapt a single-nuclei RNA sequencing protocol for frozen cancer tissue biopsy samples from another study to complete their analysis. The team showed for the first time that by using nuclei isolated from frozen livers, it is possible to identify known responses caused by TCDD exposure, as well as gain novel insight into how specific cell populations respond.

Additionally, because the single-nuclei sequencing method can analyze gene expression at unprecedented resolution, the researchers were able to identify and characterize rare cell types. This discovery further clarifies the significance of tissue spatial organization. This finding is important as it has been established that drugs and toxicants elicit spatial, or zonal, toxicities. Acetaminophen, for example, primarily affects the centrilobular region of the liver due to higher expression of certain enzymes.

Demonstration of the feasibility and value of a single-nuclei RNA sequencing approach was a critical step for applying it to more complex study designs. This improved mechanistic insight has wide-reaching

implications in the field of toxicology and beyond. This method can be applied to other parts of the human system with different contaminants.

This work expands use of single-nuclei sequencing in toxicology. Research investigating environmental contaminants, drugs, and dietary supplements can use this methodology. Better targeted therapies and risk assessment, heightened knowledge of how to prevent toxicity and disease, and improved ability to set substance thresholds and limits are all potential outcomes.

Future efforts by Nault and the team of MSU SRP Center researchers will explore the use of this technology to characterize the cell-specific sensitivity upon exposure to liver toxicants in order to better understand the development and progression of toxicant-associated nonalcoholic fatty liver disease.

If you'd like to learn more about this research, visit the Superfund Research Program website at niehs.nih.gov/srp. From there, click on the Research Brief title under the banner, and refer to the additional information listed under the research brief. If you have any questions or comments about this month's podcast, send an email to srpinfo@niehs.nih.gov.

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