MITIGATION - SCIENCE AND TECHNOLOGY ADVANCES FOR CHEMICAL AND BIOLOGICAL HAZARD MITIGATION

Leveraging Human Organoid Models And Advanced Microscopy To Probe 4D Mitochondrial Dynamics With Applications In Drug Development And Toxicology

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We aim to develop a first-of-its-kind high throughput screening platform that takes advantage of human organoid models, advanced microscopy techniques, and detailed quantitative readouts to understand the effects of chemical and biological threats on human biology.

Changes in cellular health as a result of pathogens or toxic substances are exhibited as changes in the three-dimensional mitochondrial network. This network provides 95% of the energy in the human body and regulates key cell signaling pathways, such as their response to apoptotic stressors and inflammatory mechanisms. To gain a better understanding of how this network relates to human health and to use it as a biomarker, it must be studied in 4 dimensions (4D, x,y,z,t) in living human tissue. Human stem cell derived organoid models are an accessible system where we can study subcellular dynamics at a tissue scale under specified conditions.

Lung and intestinal organoids mimic the epithelial layer of their respective organs. Typical methods for analyzing these organoids often only explore single, terminal time points and can lose spatial information. Confocal microscopy can visualize the subcellular environment, but only briefly because of its damage to the sample and rapid photobleaching effects. We use lattice light sheet microscopy (LLSM) to capture 4D mitochondria data in these organoids with low photo-toxicity and high spatio-temporal resolution. Our lab developed MitoTNT to quantify mitochondrial morphology and dynamics in the resulting datasets, so we can study network structures, remodeling rates, and diffusivity of mitochondria in each cell in live organoids.

We have investigated mitochondrial behavior as stem cells differentiate into lung and intestine organoids and have defined characteristic mitochondrial dynamics for each stage. Furthermore, we are able to predict age and organoid type based solely on mitochondrial behavior. We have observed changes in mitochondrial dynamics in the lung under toxic conditions, including exposure to polluted water and infection with SARS-CoV-2.

We combine this capability with microfluidic systems to multiplex the process. By developing a multiplexed sample chip compatible with both organoid culture and LLSM, we create the first high throughput screening platform for investigating 4D cell biology in organoids. This allows us to investigate the changing mitochondrial network in live organoids under defined conditions. Using this, we can understand not only how the energy demands of the cell change under new conditions (eg. exposure to a toxin) in its native tissue environment, but also how to guide the cell back to a healthy state with therapeutics. Having this process multiplexed decreases the required time for best therapeutics to be determined.

This platform can be used in a variety of organoid systems, such as intestine, brain, and skin, in a variety of conditions to understand how mitochondrial dynamics respond to toxic environments. Using the mitochondrial network as a proxy for cellular health and function, we can suggest treatments for specific conditions, better understand changes in cell function as a result of toxic perturbations, and test how any of this might change according to organ type.