useful to eliminate the dysfunctional mitochondria, is totally abolished.

Our results are discussed in light of the recent results, especially, the elegant work of Wang's team [3], which bridges the gap between abnormal ROS production in BTHS derived-iPSCs, and the mechanical defect of the sarcomeric structure and function.

References:

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Cell Proliferation and Death (B50)

385/B50

Nuclear Apoptotic Volume Decrease in Individual Cells: Confocal Microscopy Imaging and Kinetic Modeling

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Nuclear volume decrease of cells undergoing apoptosis (nuclear AVD) is poorly investigated. In the current work, the time-lapse live-cell microscopy imaging was performed using Leica TSC SP5 X microscope (Leica, Wetzlar, Germany), equipped with a white-light laser (WLL, 40 mW; Leica, Mannheim, Germany; 470–670 nm in 1 nm increments); 63x/1.40NA oil HCX PL APO lambda blue Objective (Leica), and a 5% CO_2, 37°C humidified incubation chamber. HepG2 cell line stably expressing H4-Dendra2 (obtained from Professor Ivan Raška, Institute of Cellular Biology and Pathology, First Faculty of Medicine, Charles University, Prague, Czech Republic) were cultivated on microscopic 50-mm glass bottom dishes (MatTek, Corporation, Ashland, MA, USA; #P50G-0-30-F), in Dulbecco's modified Eagle's Medium (PAN, Germany) supplemented with 10% fetal calf serum (PAN), 100 IU/ml penicillin, and 100 µg/mL streptomycin, in a humidified atmosphere containing 5% CO2, at 37°C.

Our results show, that the nuclear volume decrease occurs simultaneously with peripheral condensation of nuclear chromatin (forming a so called "apoptotic ring"). We proposed an osmotic model that quantitatively explains the observed nuclear AVD kinetics on the early stage of apoptosis. Fortunately, the corresponding nonlinear master differential equation of the model has an analytical solution that is useful in practical applications. Additionally, we developed an image processing method that corrects experimental images distorted by the limitation of the microscope scanning rate. The method was verified using modeled images. The developed approach was successfully applied for the treatment of the obtained experimental data in order to evaluate nuclear AVD rate parameters.

Cell Sorting and Selection (B52)

386/B52

An Accessible Live Single-Cell Detection, Sorting, and Isolation Platform

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Recent tools such as CRISPR/Cas9 have unlocked the tremendous potential of gene editing for helping treat, and even cure, devastating diseases. Access to live single-cells from biological samples is critical for gene editing workflows, cell line/antibody development, and fundamental insights into heterogeneous cancer biology. This has prompted the need for enabling tools that are widely accessible for these workflows. There is a need for a system that can effectively and efficiently process samples for enrichment, using a simplified workflow and at an affordable price, enabling adoption of systems by individual researchers at industrial/biopharma and academic sites. The Nodexus NX-One platform has been developed to address these needs for accessible live single-cell isolation workflows.

The system leverages a multiple parameter approach utilizing Node-Pore Sensing (NPS) and fluorescence detection. NPS is based on patented microfluidics that allow for highly sensitive absolute counting, sizing, and morphological measurements of cells. As a cell enters microchannel "pore," it blocks the flow of current, leading to a transient decrease in the pore's electrical current, which contains a unique detection signature. Unlike traditional Coulter sensing, NPS utilizes unique geometries to enhance detection sensitivity. NPS is used in parallel with fluorescence detection to allow for marker or fluorescent-reporter-based (e.g., GFP) selection, and the platform can be used in modes where the excitation source is not turned on, and NPS is solely used in order to minimize phototoxicity effects. These detection methods are used in combination with highly robust, low-shear microfluidic flow switching, to allow for walk-up usability and viable cell collection for downstream processing. We have demonstrated the ability to isolate target single cells, including validation with imaging and viability assays after isolation. Current results include an isolation efficiency of single cells in ~94% of wells with viability >99%.

Cell Sorting and Selection (B54)

387/B54

HIV-1 Analysis and Sorting by Virometry: A Novel Approach for Development of Virus Assays

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"Collective infectious units" are viral structures such as virion aggregates or polyploid virions, which may deliver multiple viral genome copies to the same cell. Viral aggregates have been recently described for different viral species, such as the vesicular stomatitis virus, and strongly enhance viral infectivity. Whether HIV-1 particles can aggregate, under which conditions and what are the functional consequences in terms of viral spread, has not been fully investigated so far.

Virometry is an adaptation of flow cytometry technology for the sorting of individual nanoparticles, such as extracellular vesicles (EVs) and retroviruses. In this study, we optimized cell sorter parameters for the detection of retroviral particles, sample labeling, laser power, and voltage settings. We compare 2 high speed sorters with specific configurations: The first instrument is an Astrios, which has 7 lasers jet-in-air upgraded with the EQ module to increase the detection on FSC dual PMTS. The second is a custom FACSAria III, with a high-laser power on the blue laser (488 nm), and an additional SSC detector on the violet (405nm). We use polystyrene beads (Megamix FSC and SSC, BioCytex) as an internal representative of the size profiles of the particles. Our strategy to push the limit of the conventional sorter, meaning below 0.3µm, is to use the blue laser with a high-laser power and SSC discrimination plot to increase the resolution. With specific instrument settings, we obtain a high-resolution and discrimination of beads between 0.1 and 0.3µm, but also of viral preparation.

We applied virometry to investigate if HIV-1 cell-free particles can interact together. To visualize the viral particles, we designed fluorescent virions incorporating in trans the HIV-1 Vpr-GFP protein. This approach allowed the visualization of different viral strains, both laboratory-adapted and transmitted/founder viruses, with no need of viral genome modification. Preliminary results suggest that HIV-1 virions form aggregates of different sizes, and this process correlated with increased viral infectivity.

This work will allow the use of sensitive flow virometry to sort different HIV sub-populations, and investigate their infectivity, fusogenicity, viral protein composition, and antigenicity.