Introduction

Endoplasmic reticulum (ER) mediated cell stress and the unfolded protein response (UPR) serve as major stress pathways in eukaryotic cells. ER stress and the UPR can be triggered by a number of cellular perturbations including homeostasis ER Ca2+ accumulation of mutated proteins within the lumen of the ER, and reactive oxygen species (Gozalpino et al., 2016; Schaper and Hoozemans, 2015; Zhou et al., 2016). ER stress has been implicated in a wide range of diseases including neurodegenerative diseases, cancer, diabetes and obesity (Couch, 2012; Marmur et al., 2009; Bairoch et al., 2015). Schaper and Hoozemans, 2015; Bairoch et al., 2015). Activation of proinflammatory pathways, such as the UPR and ER stress serves as an early indicator of cell stress or toxicity, which is a useful mechanism to detect drug or compound induced cell stress (Doezema et al., 2012; Trouche and Fromenty, 2015).

This UPR consists of three arms, each activating a unique mechanism to regulate the cellular response to ER stress (Gozalpino et al., 2016). Methods to detect activation of ER stress and the UPR range from FCR-based assays to fluorescent indicators (Kawasaki et al., 2004; Bao et al., 2015). However, live-detection of ER stress are optimized for detecting both chemical as well as genetically induced stress, and are irreversible indicators. Demonstrated here is the use of a new and reportedly available ER stress sensor developed by Montana Molecular to detect chemically induced cell stress by thapsigargin, a potent SERCA pump inhibitor that induces ER stress using the Agilent BioTek Lionheart FX automated microscope allows the detection of both the initial and recovery from thapsigargin induced cell stress as well as simultaneous detection of complemented cell signaling over a 24-hour period. In sum, combining live cell detection of stress and signaling with levels high-cost monitoring allows precise detection of changes in cellular stress levels and its implications on cellular signaling events.

Agilent BioTek instrumentation

Agilent BioTek FX automated microscope with dual reagent injector module and augmented microscopy

All inclusive microscopy system: Optimized for brightfield imaging with high resolution color imaging and fluorescence channels. Up to 10x air and immersion magnification.

Up to 20 frames per second (fps) image capture and dual in-line reagent injectors: Allows capturing up to 20 frames per second (fps) of data for both channels of imaging, allowing for rapid image analysis and kinetic cell counts.

In-stream environmental control: Conditions for long-term imaging of live cells.

Integrated environmental control: Incubation up to 40 °C with CO2, O2, and available humidity, providing optimal conditions for long-term imaging of live cells.

Powerful Agilent BioTek FX microscope and image software: Automated image capture, processing, and analysis tools, including dual masking for cell counting and subpopulation analysis, plus annihilation and movie marker functions.

Methods

Live-cell imaging of cell stress and GPCR activity using Montana Molecular biosensors

Results and discussion

Automated imaging-based quantification of treatment-induced cellular stress

Ratiometric detection of cellular stress and toxicity using expressed biosensor

Figure 6. Thapsigargin-induced cellular stress was monitored using the cell stress sensor. The ratio of green (cell stress sensor) to red (nuclear marker) fluorescence intensity was calculated to account for variations in cell number across different conditions. A kinetic profile of the green to red fluorescence ratio revealed that peak cellular stress was reached approximately 6 hours after thapsigargin treatment, with a greater than two-fold increase in the cellular stress response over baseline levels. Although ratiometric detection of cellular stress provides a valuable endpoint metric for evaluating the level of induced stress in a cell population, changes in the expression level of the nuclear marker over time can lead to kinetic profiles that are difficult to interpret. This shows that stress levels appear to drop below baseline due to a steady increase in the expression level of nuclear marker over the 24-hour period.

Monitoring Gq-dependent Ca2+ release using R-GECO biosensor and automated image analysis

Figure 7. R-GECO expressing the R-GECO calcium biosensor were used to measure release of intracellular calcium resulting from Gq-coupled H1 receptor stimulation. Image panel of untreated HEK293 cells (A) with Agilent BioTek Gen5 placed masks around cells containing R-GECO fluorescence (B). R-GECO fluorescence – which increases with increasing levels of Ca2+ – is initially low before pretreatment. After stimulation of Gq coupled H1 receptors by injection of 30 µM (red) carbachol, rapid intracellular mobilization of Ca2+ and a corresponding increase in R-GECO fluorescence is observed, followed by a gradual decrease in cytoplasmic Ca2+ levels.

Figure 8. Kinetic monitoring of R-GECO fluorescence reveals calcium signaling kinetics are altered by thapsigargin in a dose- and treatment duration dependent manner. HK293 expressing H1 receptor and the R-GECO calcium sensor were treated with thapsigargin, a potent SERCA pump inhibitor, to evaluate the long-term effects of thapsigargin on Gq-dependent calcium signaling. Carbachol (10 µM final) was added to the cells as the ligand to trigger the calcium response. The percentage of cells exhibiting R-GECO fluorescence was counted at each time point, with the use of Gen5 subpopulation analysis. (A) The kinetic profile of the percent stressed cells provides a more detailed analysis of the calcium signaling. (B) Multiplexing cell stress and R-GECO biosensors to interrogate calcium signaling within stressed and unstressed cells within a population.

Multiplexing cell stress and R-GECO biosensors to interrogate calcium signaling within stressed and unstressed cells within a population.

Conclusion

- Demonstrated here is the ability to monitor cell stress in live cells and in real time using a novel expressed biosensor, while simultaneously examining the effects of cell stress on second messenger signaling.
- Cell stress induced by thapsigargin is detected on an order of magnitude prior to any changes in cellular proliferation.
- Image-based cellular analysis enables unique quantitative evaluation of cell stress, including percentage of cells within the population exhibiting stress over time.
- Calcium signaling kinetics monitored using the R-GECO biosensor are altered by thapsigargin treatment in a dose- and time dependent manner.
- Subpopulation analysis reveals that cells exhibiting high levels of stress response have considerably different prestimulation and poststimulation levels of calcium compared to cells exhibiting lower levels of stress.

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