High-Content Imaging Detection of Cellular Lipids to Measure Lipid Toxicity and Cell Differentiation

Abstract

The detection and understanding of preclinical mechanisms in toxicological profiling and compound screening are important components of drug discovery. Cellular lipid accumulation resulting in intracellular accumulation (phospholipidosis) or extracellular lipid droplets or lipid peroxidation can be qualitatively detected in real-time using Thermo Scientific® Dyes. The dyes are well-suited to automated, cell-based imaging analysis. They are brighter than competitor’s products and give robust assay performance with high Z’ factors (>0.7). They are specific and sensitive and validated for quantitative measurements in high-content imaging assays for adipogenesis cell targets. A comparison with other phospholipid probes demonstrated that the Thermo Scientific® Red Fat Dye results in stronger droplet staining as compared to other probes.

Introduction

Early sensitivity detection of a substance and identification of the toxic mechanism are of great value during drug development. Automated, quantitative cellular imaging (e.g., high-content imaging) is a powerful assay method for predicting compound toxicity. Detection and determination of induced cellular toxicity can be extended by multiple markers that indicate cellular responses to a variety of drug classes. Adipogenesis detection with Thermo Scientific® Red Fat Dye is a simple tool for detecting the cellular responses to toxic compounds such as fatty acids or neutral lipids. However, the detection of cellular lipid accumulation requires a specific and sensitive imaging assay. We have also developed fluorescent probes for monitoring phospholipid accumulation in mammalian cells that can be used in high-content imaging (Thermo Scientific® Phospholipid Green & Orange) and in real-time lipid droplet imaging (Thermo Scientific® Red Fat Dye) without the need for metabolic labeling.

Key cellular responses depend on fluorescent probes that enable specific and sensitive detection of phospho- and neutral lipids in mammalian cell lines. The phospho- and neutral lipid probes were designed to test the specific lipid probe detection of phospholipids or neutral lipids. The reagents were tested in a steatosis lipid toxicity model (498/532 nm) or orange (557/584 nm) fluorescence emission. Neutral lipids were detected with high specificity using a specific probe with red fluorescence emission (ex/em: 580/610 nm) on live or fixed cells without wash steps. The phospho- and neutral lipid probes were developed to specifically stain phospholipids or neutral lipids depending on the cellular compartmentalization in the cell. We found that the cellular lipid detection reagents were specific for their respective targets by their control cell lines. Detection was further confirmed in HepG2 cells treated with a heptane model of steatosis. A combination of probes for phospholipids and anti-adipogenic drugs is needed for the evaluation of neutral lipids. The lipid droplet staining was not specific for all cellular structures and was able to detect the lipid droplet staining in the cytoplasm of mammalian cell lines after the incubation of test compounds. We developed a set of fluorescent reagents for modified, cell-based, high-content imaging experiments to automatically detect and quantify phospholipids. The new detection reagents label the accumulation of phospholipids in live or fixed cells and have either green (498/532 nm) or orange fluorescence emission. We have also developed a fluorescent drug, Red Fat Dye (498/532 nm) to specifically stain neutral lipids in cells. The reagents show a high efficiency and specificity for neutral lipid droplets in live and fixed cells and are compatible with our phospholipid detection reagents. These data demonstrate that specific detection of lipid accumulation and compound screening are important components of drug discovery. Cationic overloading in the liver and adipose tissue can occur in normal and pathological conditions; e.g., to study the toxic effect of fatty acids or neutral lipids.

Conclusions

Thermo Scientific® Phospholipid Orange and Green reagents:

• Are well-suited to automated, cell-based imaging analysis.

Thermo Scientific® Red Fat Dye:

• Label neutral lipid droplets that occur in steatosis and adipogenesis.

• Give robust assay performance with high Z’ factors (>0.7).

• Are well-suited to automated, cell-based imaging analysis.

Neural Lipid and Phospholipid Co-Detection

Neural Lipid and Phospholipid Co-Detection

HepG2 cells were treated with 30 µM cyclosporin A, 48 hrs. The cells were fixed with Thermo Scientific® 4% paraformaldehyde for 48 hrs. The cells were then treated with Hoechst 33342. Dye performance was assessed after image acquisition using a Cellomics ArrayScan® HCS Reader. EC50 values were determined using the same exposure time, image integration, and Z’ factor analysis. The assay produces robust performance: Thermo Scientific® Phospholipid Green & Orange, EC50 = 18.5 µM cyclosporin A (1.01 ± 0.09, Z’ = 0.83 ± 0.07), and Thermo Scientific® Phospholipid Orange, EC50 = 20.4 µM cyclosporin A (1.01 ± 0.09, Z’ = 0.83 ± 0.07).

Neural Lipid and Phospholipid Co-Detection

Adipogenesis Detection with Thermo Scientific® Red Fat Dye

HepG2 cells were treated with cyclosporin A and 0.5% fatty acids, or left as control. Images were automatically acquired and quantitatively analyzed by the Thermo Scientific® Cellomics® HCS Reagents & Kits, Comparative Measurements ToolBox. Thermo Scientific® Red Fat Dye results in stronger droplet staining as compared to other products.

Comparison with Other Phospholipid Probes

HepG2 cells were treated with 30 µM propranolol for 48 hours, or left as control. Nuclei were counterstained with Hoechst 33342. Dye performance was assessed after image acquisition using a Cellomics ArrayScan® HCS Reader. EC50 values were determined using the same exposure time, image integration, and Z’ factor analysis. The assay produces robust performance: Thermo Scientific® Phospholipid Green & Orange, EC50 = 18.5 µM propranolol (1.01 ± 0.09, Z’ = 0.83 ± 0.07), and Thermo Scientific® Phospholipid Orange, EC50 = 20.4 µM propranolol (1.01 ± 0.09, Z’ = 0.83 ± 0.07).