Simultaneous analysis of cell death mechanisms and oxidative stress using Molecular Probes® next generation imaging reagents and flow cytometry

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ABSTRACT

Cell death can occur through multiple pathways, such as apoptosis, autophagy, and necrosis. Although necroptosis for proper growth and development, dysfunction of apoptosis has been associated with a variety of diseases including cancer, Alzheimer’s disease, and neurodegenerative diseases. Increased oxidative stress has also been associated with these diseases and has been shown to lead to apoptosis and autophagy. Importantly, cell death can occur through a single pathway, or in concert with multiple pathways. Staurosporine has been shown to induce apoptosis, chloroquine is known to promote autophagy, and necrostatin results in both apoptosis and autophagy. In this study we utilized multi-parametric high content imaging and flow cytometry to differentiate between apoptotic and autophagic cell death after induction by different agonists. In addition, we simultaneously examined levels of oxidative stress to determine the relationship between oxidative stress and cell death. We used the fluorescent CellEvent™ Caspase-3/7 Green Detection Reagent as an indicator of apoptosis, LC3B RFP and an antibody specific for LC3B to measure autophagy, and CellROX™ Deep Red Reagent, a near infrared fluorescent ROS probe to evaluate oxidative stress. Furthermore, loss of mitochondrial membrane potential was also observed in apoptotic cells. By using a multi-parametric approach, high content imaging or flow cytometry we were able to characterize the mechanism of cell death by discriminating between cells which were apoptotic (active caspase 3/7), autophagic (LC3B positive autophagosomes), or both. This multi-parametric approach provided detailed information about cell survival and correlation between oxidative stress and different mechanisms of cell death.

INTRODUCTION

Increased oxidative stress has been associated with a variety of diseases including cancer, Alzheimer’s disease, and other neurodegenerative diseases.

High levels of oxidative stress can lead to cell death through several mechanisms including apoptosis and autophagy.

Understanding the relationship between oxidative stress and cell death is essential to our understanding of disease progression and the development of therapeutic interventions.

Live cell microscopy allows temporal resolution between cellular events such as increased oxidative stress and apoptosis signaling.

Multi-parametric imaging allows for several parameters to be analyzed simultaneously within the same cell providing key information at the cellular level.

Figure 1: Validation of probes used for apoptosis, oxidative stress, and autophagy by fluorescence microscopy

RESULTS

Figure 2: Multiplex imaging of oxidative stress and activated caspase 3/7

Figure 3: Multiplex time-lapse imaging showed loss of mitochondrial membrane potential followed by caspase 3/7 activation

Figure 4: High content imaging reveals progression of apoptosis and autophagy. HeLa cells were treated with 0.5 µM staurosporine for 0 – 4 hours in the presence of 20 µM Z-DEVD-FMK caspase inhibitor (red) and 7.5 µM CellEvent™ Caspase-3/7 Green Detection Reagent (green). Cells were then stained with 5 µM CellROX™ Deep Red Reagent and Hoechst 33342 for 30 minutes at 37°C, then washed with warm DPBS. Images were acquired and analyzed on a Thermo Fisher Scientific Arrayscan® VTI. Increased oxidative stress was observed at 0.5 hour after treatment (magenta) while caspase 3/7 activation was not observed until 4 hours after treatment (green) as shown in representative cells above.

Figure 5: Simultaneous detection of apoptosis and autophagy by fluorescence microscopy

Figure 5A-B: HeLa cells treated with staurosporine undergo apoptosis and autophagy: HeLa cells were treated with 0.5 µM staurosporine for 0 – 4 hours through 30 minutes in the presence of 7.5 µM CellEvent™ Caspase-3/7 Green Detection Reagent (green). Cells were stained with 5 µM CellROX™ Deep Red Reagent and Hoechst 33342 for 30 minutes at 37°C, then washed with warm DPBS. Images were acquired and analyzed on a Thermo Fisher Scientific Arrayscan® VTI. Signal intensity for caspase 3/7 (nuclear) and LC3B (spots within the cytosol) were plotted as fold change relative to 0 hour treatment for each of the two parameters (B). Staurosporine significantly increased caspase 3/7 activation after 4 hours, which was associated with a small increase in autophagosome formation.

A rapid and easy-to-use caspase 3/7 fluorescent reagent is demonstrated in imaging and flow cytometry for detection of apoptotic cells.

HeLa cells were treated with PARP inhibitor to increase oxidative stress followed by activation of caspase 3/7. Loss of mitochondrial membrane potential was also observed indicating cell death occurring by apoptosis.

In U-2 OS cells chloroquine induced a significant increase in autophagosome formation with minimal change in caspase 3/7 activity.

By using fluorescent markers for oxidative stress, active caspase 3/7 and LC3B it was possible to determine the role of oxidative stress relative to activation of caspase 3/7 and distinguish between cells which were apoptotic or autophagic.

This multi-parametric approach was also amenable to time lapse imaging which enabled temporal resolution of oxidative stress relative to cell death mechanisms.

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