Rapid quantification of autophagy in cell-based assays

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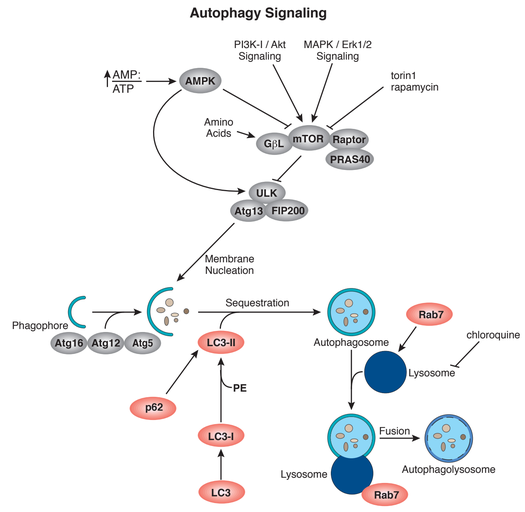
Abstract

Chemically induced upregulation of autophagy has gained importance as a potential prophylactic or therapeutic treatment for numerous diseases, particularly neurodegenerative disorders. Cell-based drug screening to identify pro-autophagic compounds often relies on time-consuming microscopic imaging and high-resolution analysis. Though fast laser-scanning platforms exist, it was unclear whether induction of autophagy could be reliably quantified using these instruments. In this study, both microscope-based and laser-scanning high content imaging platforms were used to analyze cells treated with reported autophagy-inducing chemicals. Antibodies to LC3B, Rab7, and SQSTM1/p62 were used as endpoints. The laser-scanning platform adequately quantified induction with LC3B and Rab7 endpoints, using 16% of the scan time required by the imager. However, microscope-based analysis was needed to quantify the subtle subcellular translocation of SQSTM1/p62. This study indicates that if a robust endpoint is used, high speed laser-scanning instruments are sufficient for the measurement of autophagy upregulation.

Introduction

Autophagy, the cell-based degradation of its proteins and structures, is necessary for clearing waste, balancing protein levels, and providing nutrients and amino acids to the cell. When the process is disrupted, buildup of protein waste can impede proper cell function and lead to disease states. Increased effort is being made to identify chemicals that upregulate autophagy in dysfunctional cells, thus clearing or preventing protein accumulations.

Cell-based high content screens are an effective means of identifying lead compounds. Image-based analysis offers relatively high resolution but at the expense of throughput speed. Laser-scanning cytometers lack the resolution to differentiate cytoplasmic organelles, but provide fast whole-well quantification. This study was conducted to determine whether the subcellular translocation of proteins associated with autophagy could be adequately quantified on a laser-scanning platform.

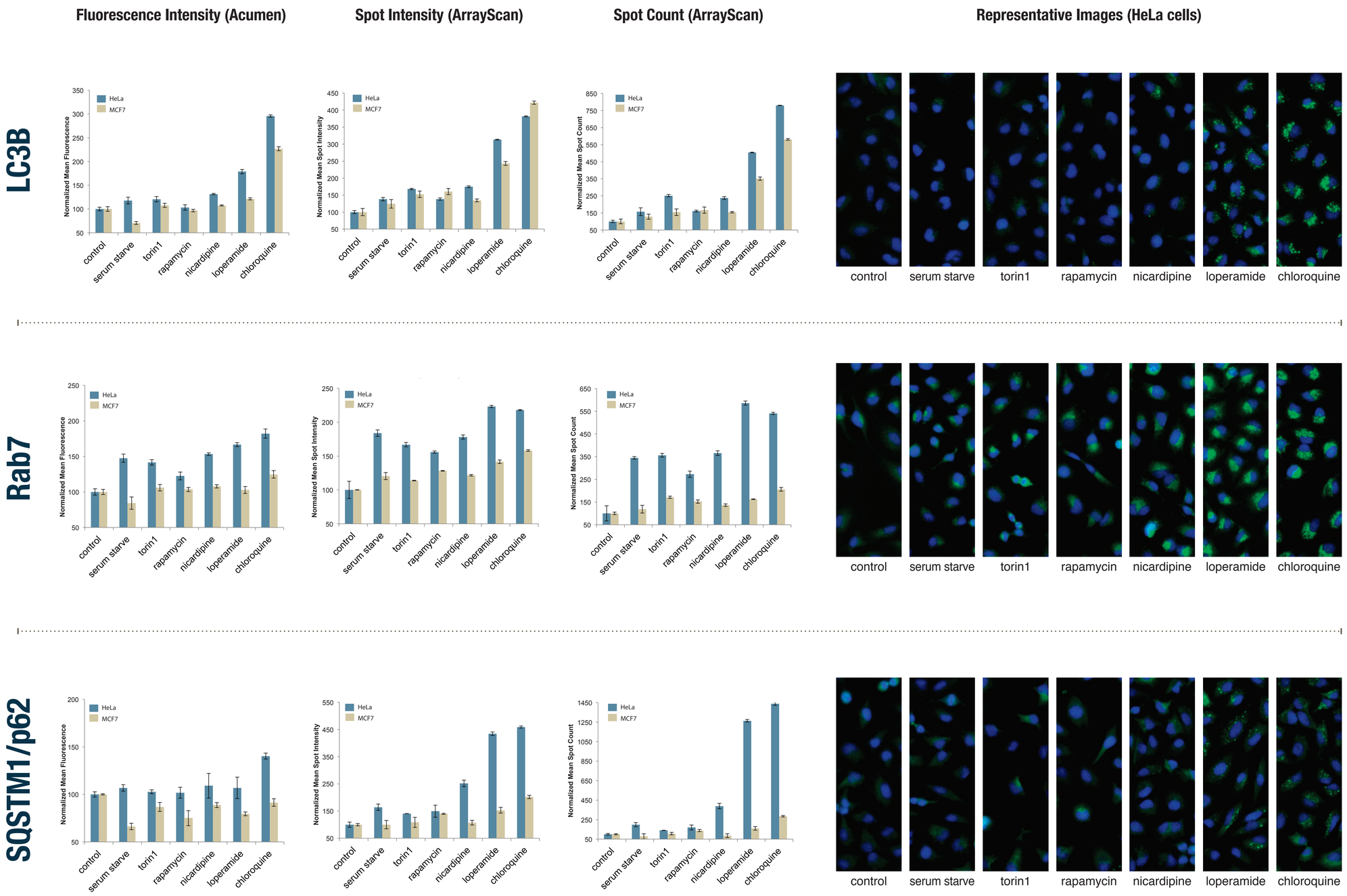


Methods

HeLa cells and MCF7 cells were grown in 96-well plates under normal culture conditions for 24 hours, prior to serum starvation or drug treatments. For serum starvation treatment, media was removed and replaced with serum-free media containing 0.1% DMSO. For drug treatments, media was removed and replaced with serum-free media containing drug at concentrations indicated below. Control wells received full media with 0.1% DMSO. Following treatments, cells were fixed with ice cold 100% methanol and blocked with 5% normal goat serum. Antibodies to LC3B, Rab7, and SQSTM1/p62 (Cell Signaling Technology (CST), #3868, #9367, and #7695) and anti-rabbit Alexa Fluor® 488 conjugate (CST #4412) were added according to the CST recommended immunofluorescence protocol. Nuclei were labeled with propidium iodide and Hoechst 33342. Imaging of at least 1000 cells/well was performed using a Cellomics® ArrayScan® V^{TI} using a 20x objective, and quantification was performed using the Spot Detector assay. Cell-level fluorescence quantification in a 6X2 mm area (roughly 3500 cells/well) was performed with a TTP LabTech Acumen® laser scanning cytometer, at 4X1 μm resolution. Fluorescence intensities and spot counts were normalized to control wells.

Drug treatments (2 hrs):
chloroquine, 50 μM;
loperamide hydrochloride, 4.9 μM;
nicardipine hydrochloride, 4.8 μM;
rapamycin, 10 nM;
torin1, 250 nM.

Results



Conclusions

- Laser-scanning imaging cytometers sufficiently quantify upregulation of autophagy, as detected by LC3B and Rab7 antibodies
- Screening throughput is much higher on laser-scanning platforms
  - 8 min/plate on Acumen®, quantifying ~3500 cells/well
  - 50 min/plate on ArrayScan® V^{TI}, quantifying 1000 cells/well
- MCF7 cells show lower induction of autophagy, and serum starvation may decrease basal level of autophagy
- Longer treatment time may be required for less potent inducers, such as rapamycin and torin1
- Proteins which undergo subtle translocation, such as SQSTM1/p62, may require high-resolution imaging for accurate measurement

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