Easy Screening of Autophagy-Associated Acidic Compartments with the Sapphire Biomolecular Imager

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Introduction

Autophagy is the homeostatic cellular mechanism by which unneeded cellular components are degraded by lysosomes. Disruption of the autophagy pathway contributes to the development of a variety of human diseases. Due to its key role in several cellular processes, autophagy is a double-edged sword that can induce death or survival of cells in a context-dependent manner (1,2). Mechanistically, the autophagic pathway includes the formation and maturation of a series of different acidic vesicles that eventually converge in the lysosome for cargo degradation (3,4).

Acridine orange (AO) is a cell-permeable fluorescent dye that accumulates in acidic compartments such as lysosomes and autophagic and endosomal vesicles, allowing their detection in live cells (5). As the concentration of AO increases, its fluorescence undergoes a metachromatic shift, changing from green at low concentration in the cytosol to red at high concentration within acidic vesicles (6,7).

There are several techniques and strategies for estimating autophagy, though most of them are cumbersome and not compatible with in vivo experimental designs (8). Staining vesicles with AO is an easy protocol that can be used in live cells but has a poor specificity for autophagy (8). Keeping in mind the metachromatic properties of AO, recently Thomé et al have described an improvement in the use of AO for autophagy activity detection. The ratio of the intensities of the green and red fluorescence emission of AO was found to have greater specificity for autophagy than the intensity of red fluorescence alone, and the ratio was not affected by binding of AO to nucleotides or by changes in cell size (7).

In this application note, we describe screening for autophagy activity in cells stained with AO acidotropic fluorescent dye and detected with the Azure Biosystems Sapphire™ Biomolecular Imager.

Methods

Cells from a pancreatic cancer cell line, Panc-1 (ATCC CRL-1469), were grown in 24-well plates. Cells were submitted to starvation as a physiologic induction of autophagy. After a 5 min incubation with AO, cells were imaged live in the Sapphire™ Biomolecular Imager. Imaging was done in the red channel (520/566BP24) and the green channel (488/518BP22). Regions of interest were identified in TIF images captured by the Sapphire and quantified using ImageJ 1.54f.

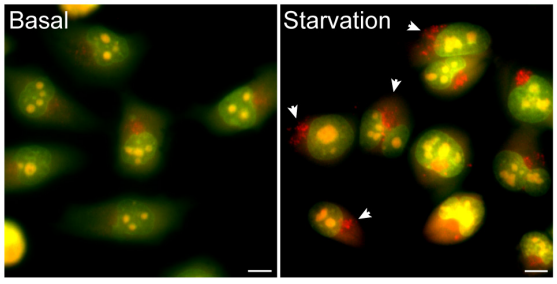

Figure 1. Merged green and red channels of live Panc-1 cells pre-incubated with 1 µg/ml AO. Arrows point to AO-stained acidic compartments apparent after 1 hour of starvation (right panel). Imaging was done in an EVOS™ M7000 Imaging System using Green (excitation 470/22 nm, emission 525/50 nm) and Red (excitation 585/29, emission 628/32 nm) cube filters.

Results and Discussion

Fluorescent imaging of the AO-stained cells in the red and green channels shows an increase of acidic compartments (fluorescing red) in response to 1 hour of starvation (arrows, Figure 1). We adapted the approach of Thomé et al (7) for use with the red channel (520/566BP24) and the green channel (488/518BP22) of the Sapphire Biomolecular Imager. After evaluation of different AO concentrations and excitation laser intensity conditions for image acquisition with the Sapphire, we determined the optimal AO concentration was 1 or 2 µg/ml and the optimal intensity settings were 1 for the red channel (520 nm laser) and 4 for the green channel (488 nm laser). These scan parameters were used in an analysis of the ratio of green to red fluorescence intensity (G/R FI ratio) in cells submitted to starvation for 1, 2, or 3 hours, using AO at concentrations of 1 µg/ml or 2 µg/ml. The greatest changes in fluorescence associated with autophagy were detected when using 1 µg/ml of AO and after 3 hours of starvation (Figure 2). Finally, treatment with the lysosome acidification inhibitor chloroquine prevented the changes in AO fluorescence in response to starvation, demonstrating the specificity of this technique (Figure 3).

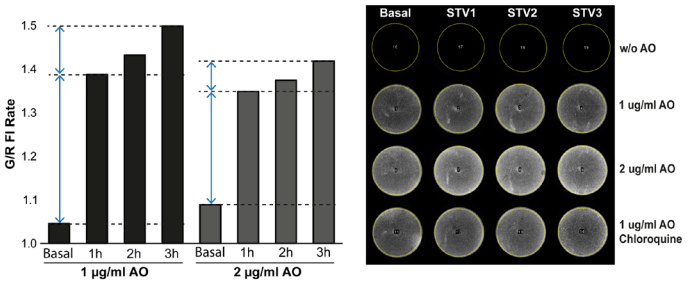

Figure 2. Green to red fluorescence intensity ratio for cells submitted up to 1, 2, or 3 hours of starvation with 1 or 2 µg/ml AO (left). Image of multi-well plate with cells subjected to 1, 2, or 3 hours or starvation (STV1, STV2, STV3 respectively), stained with 1 or 2 µg/ml AO, and incubated with or without Chloroquine (right).

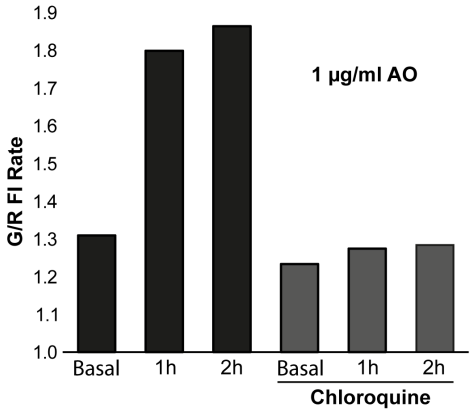

Figure 3. Green to red fluorescence intensity ratio for starved cells with or without chloroquine, an inhibitor of lysosome acidification.

In conclusion, with its ability to carry out multiplex fluorescence imaging, and to image multi-well plates including 24-well plates, the Azure Biosystems Sapphire™ Biomolecular Imager can be part of a quick and easy in vivo evaluation of autophagy induction in cell culture using this AO staining approach.

References

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