Examining the Potency of Potential Autophagy Inducing Drugs

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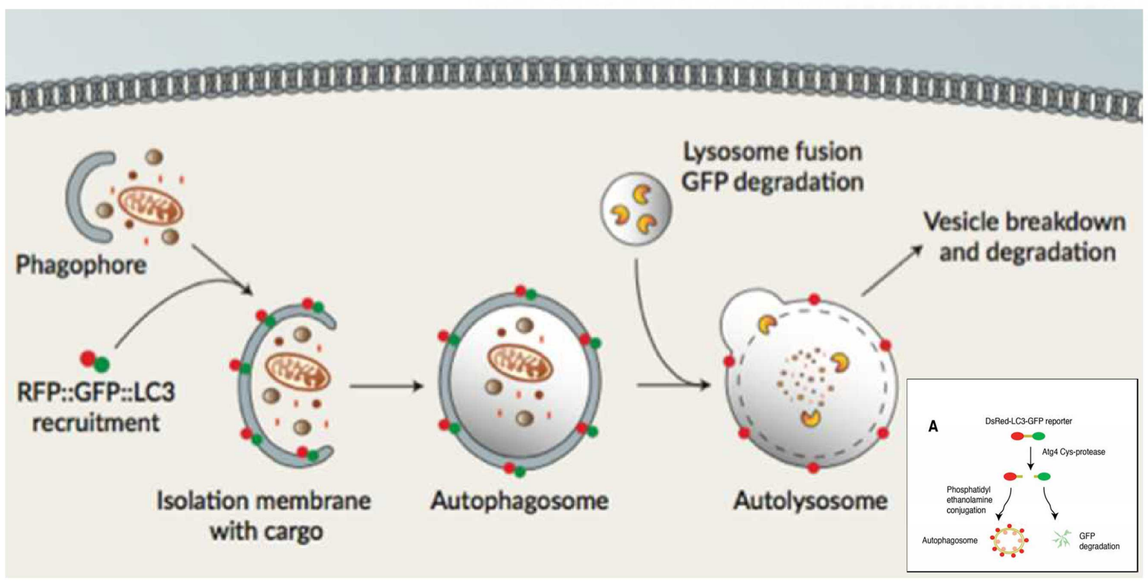
Abstract

Autophagy is the process in which a cell degrades redundant or damaged components caused by various stresses within the cell. This important homeostasis mechanism plays an important role during viral and bacterial infection. With the aim of developing a new drug to fight tuberculosis infections, we zoned in on autophagy as the main mechanism of interest. In this work, we employed confocal fluorescence microscopy to visualize the short term and long term effects of three drugs that were developed as autophagy inducers. We found that each of the drugs showed evidence of autophagy induction at different timepoints, suggesting a different mechanism of action for each.

Introduction



Autophagy induction occurs with a decrease in GFP signal. Thus, we're looking for an increase in RFP:GFP puncta ratio.

Methods

- HeLa RGL1 Medium Sort cells were cultured at 37° C with 5% CO_2
- The samples were created by adding 15µM of compounds 108, 115, and 903 as each treatment group. A 0.1% DMSO treated group is used as vehicle control.
- Images were taken at 4 and 18 hours after treatment using an Leica DMi8 DLS confocal fluorescence microscope.

Quantitative Image Analysis with In-house Written MatLab Codes

Merge & Flatten Tiffs — Flatten the 68-stack tiffs to easily identify cells in the image

→

Cell Finder — Identify the outline of individual cells within the images.

→

Z-Stack Viewer — Quantify the number, area, and intensity of the puncta in each cell

Microscopy

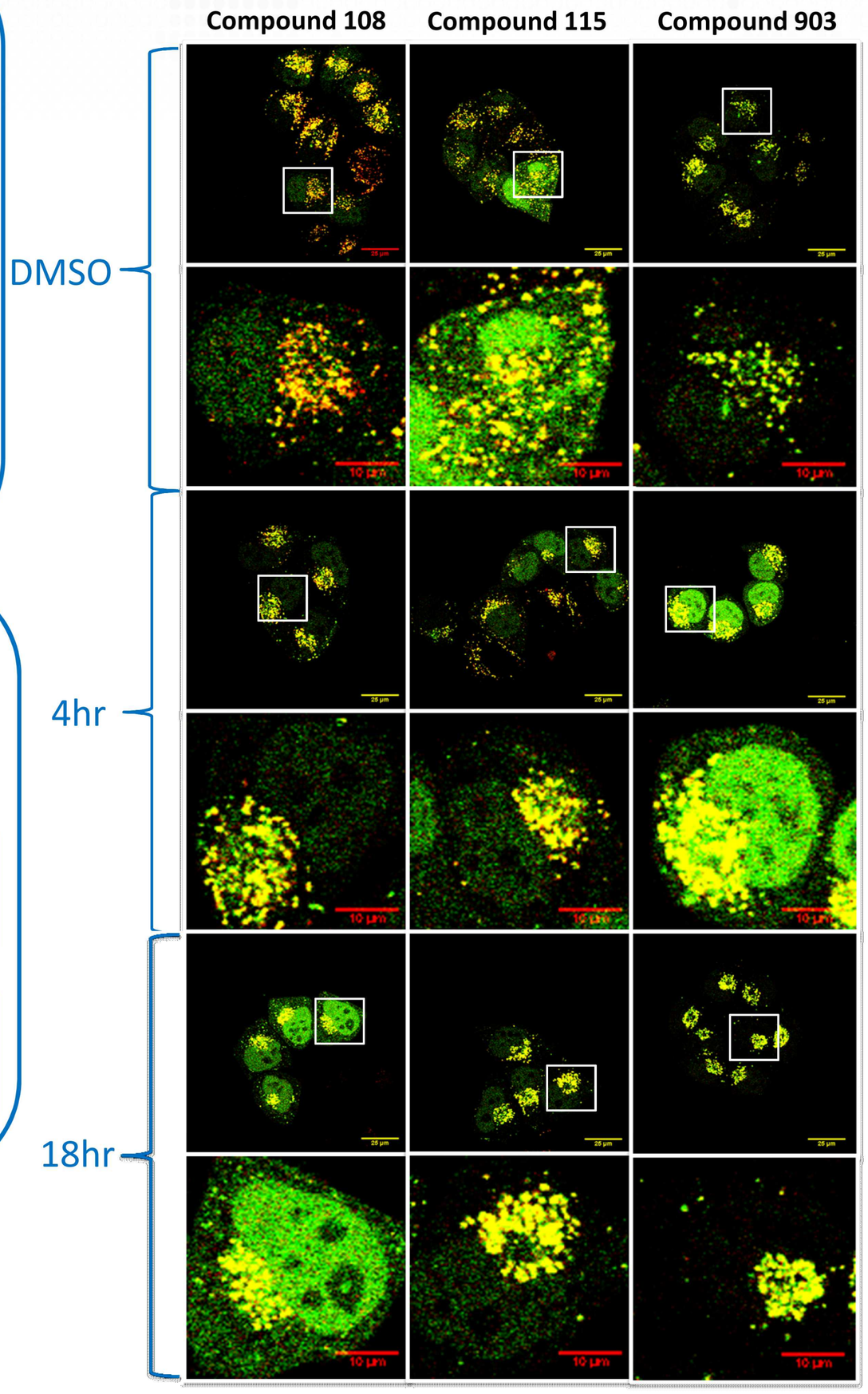


Figure 1. Image Panels
The composite and zoomed-in images (area in white box) of each treatment for each compound. The most in-focus stack of a representative image was chosen. The images is a merge between the GFP channel overlayed in green and RFP in red. All the images are shown at the same intensity and contrast levels.

Results

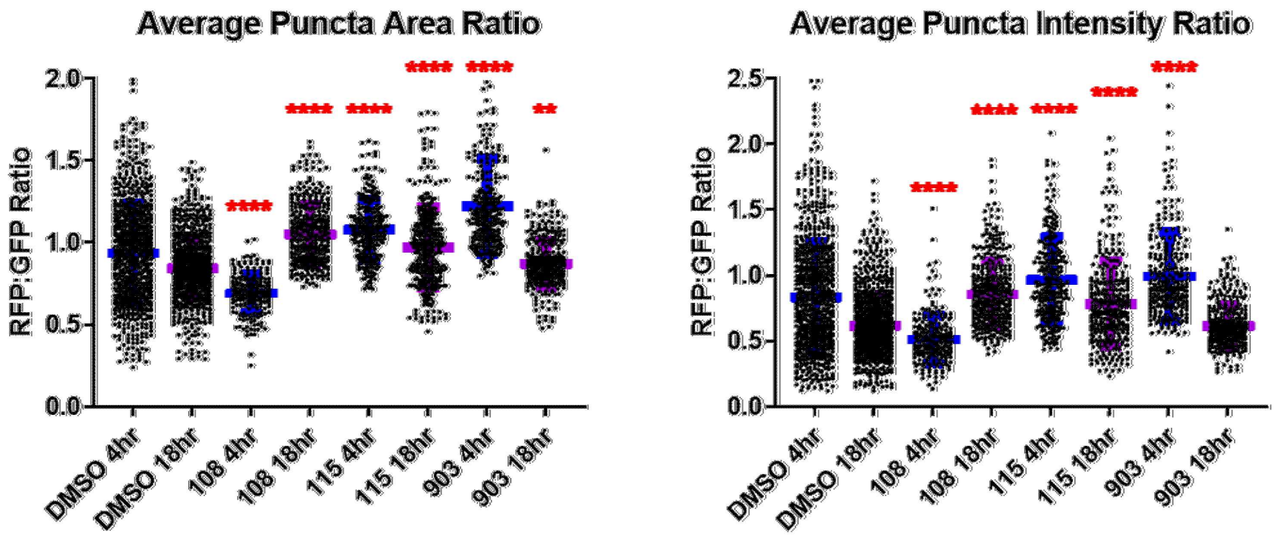


Figure 2. Graph Analysis
4hr and 18hr RFP and GFP puncta analysis of compound 108, 115, and 903 compared to DMSO as control. Statistical significance of treatment versus DMSO: ns ($p > 0.05$); * ($p \leq 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.001$); **** ($p \leq 0.0001$).

- Compound 108 showed a significant decrease in the average area and intensity of RFP:GFP ratio at 4 hours. However, at 18 hours, RFP:GFP ratios increased significantly, suggesting that this compound is a good autophagy inducer at 18 hours.
- Compound 115 showed an increase in RFP:GFP average area and average intensity ratios at both 4 hours and 18 hours. This suggests that compound 115 is able to induce autophagy early and sustain that induction through 18 hours.
- Compound 903 showed significant increase in RFP:GFP ratios at 4 hours, but wasn't as significant at 18 hours. This suggests that compound 903 induces autophagy early but is unable to continue that response at 18 hours.

Discussion

- The results suggests that each compound is acting differently within the cell. There is evidence of autophagy for all the compounds based on the graphs.
- Although little red signal is seen in the images, the yellow puncta in the R/G overlay images indicates both RFP and GFP are present. Qualitatively, the image panels show higher saturation and intensity of yellow puncta in the 4hr and 18hr treatment groups and this is verified quantitatively by increased RFP:GFP ratio that we calculate in the single cell analysis.

Future Directions

- Monitor each treatment over a 24-hour period to identify the initial autophagy induction timepoint for each compound.
- Look for possible mechanistic pathways of how each of the compounds is affecting the cells

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