Subcellular Localization and Quantitation of p62 and TFEB via High-Content Imaging

OVERVIEW

High-content imaging (HCI) is a versatile technique which allows the user to detect the presence and/or location of a desired cellular component or analyte within a cell. Here, we used a multiplex HCI staining workflow to evaluate the effects of Bafilomycin A1 and ML-SA5 treatment on the subcellular distribution of p62 and TFEB (respectively) in HeLa cells.

BACKGROUND

Bafilomycin A1 (BafA1) is a macrolide antibiotic isolated from Streptomyces species and is a specific vacuolar H^+ ATPase inhibitor. Inhibiting this H^+ pump prevents the acidification required for lysosome function. This effect makes BafA1 a frequently used tool for studying autophagy and endosomal acidification. Here, BafA1 was used as an autophagy inhibitor to demonstrate the effect of lysosome disfunction on the accumulation of p62, a protein which binds and shuttles waste to the lysosome and is typically degraded with waste. (1)

ML-SA5 is a potent small molecule agonist of transient receptor potential mucolipin 1 (TRPML1). TRPML1 is a major calcium release channel on the lysosomal membrane and plays an important role in lysosomal signaling during nutrient deprivation. Release of calcium from the lysosome by TRPML1 promotes dephosphorylation of TFEB (a transcription factor regulating the autophagy-lysosome pathway) by the phosphatase calcineurin, resulting in TFEB nuclear localization.(2) In this study, ML-SA5 was used to examine the effect of TRPML1 agonism on TFEB subcellular localization.

METHODS

Cell plating (Day 0): HeLa cells were added to a 384-well PE Opti-plate at 2,500 cells/well in 40uL full growth medium and allowed to grow overnight.
Treatment (Day 1): 11-point dose responses of BafA1 (final 0.08nM-500nM) (EMD Millipore, Cat #: 1PCX15UG) and ML-SA5 (final 0.17nM-10μM) (MedChemExpress, Cat #: HY-152182) were prepared at 2X of the final concentration, with a dilution factor of 1:3. 40uL of each dilution point was added to the cell plate. Cells were incubated for 24hr with BafA1 treatments or 4hr with ML-SA5 treatments (37°C).
Staining (Day 2-3): After treatment incubations, cells were fixed with 4% PFA. For staining, cells were first incubated with anti-p62 mouse (BD Transduction Laboratories, Cat #:610833, at 1:400) and anti-TFEB rabbit (Cell Signaling Technologies, Cat #: D207D, at 1:500) primary antibodies, followed by staining with goat anti-mouse Alexa Fluor™ 488 (Invitrogen, Cat #: A11001, at 1:1000) and goat anti-rabbit Alexa Fluor™ 647 (Invitrogen, A21244, at 1:1000) secondary antibodies and Hoechst nuclear stain. All antibody binding/staining steps were performed in block-perm buffer (1:1 Odyssey buffer/PBS containing 0.1% Triton X-100).
Imaging: Images were acquired using the GE IN Cell Analyzer 6500 HS high-content imager, using both a 20X and 40X objective lens. Analysis was performed using images taken at 20X, representative images (right) were taken at 40X.
Masking Strategy: Masking was done using the Molecular Devices IN Carta software. Hoechst (nuclear stain) was used to mark nuclei. Green/AF488 (p62 stain) was used to define the cell body (image panel a-c at right).
Analysis: To quantitate changes in cytoplasmic p62 accumulation, the average green intensity of the cytoplasm per cell was plotted as a function of dose (image panel d-f at right). To assess TFEB subcellular localization, the ratio of cytoplasmic to nuclear AF647/red intensity was plotted as a function of dose (image panel g-i).

HIGH-CONTENT IMAGING MASK AND RESULTS

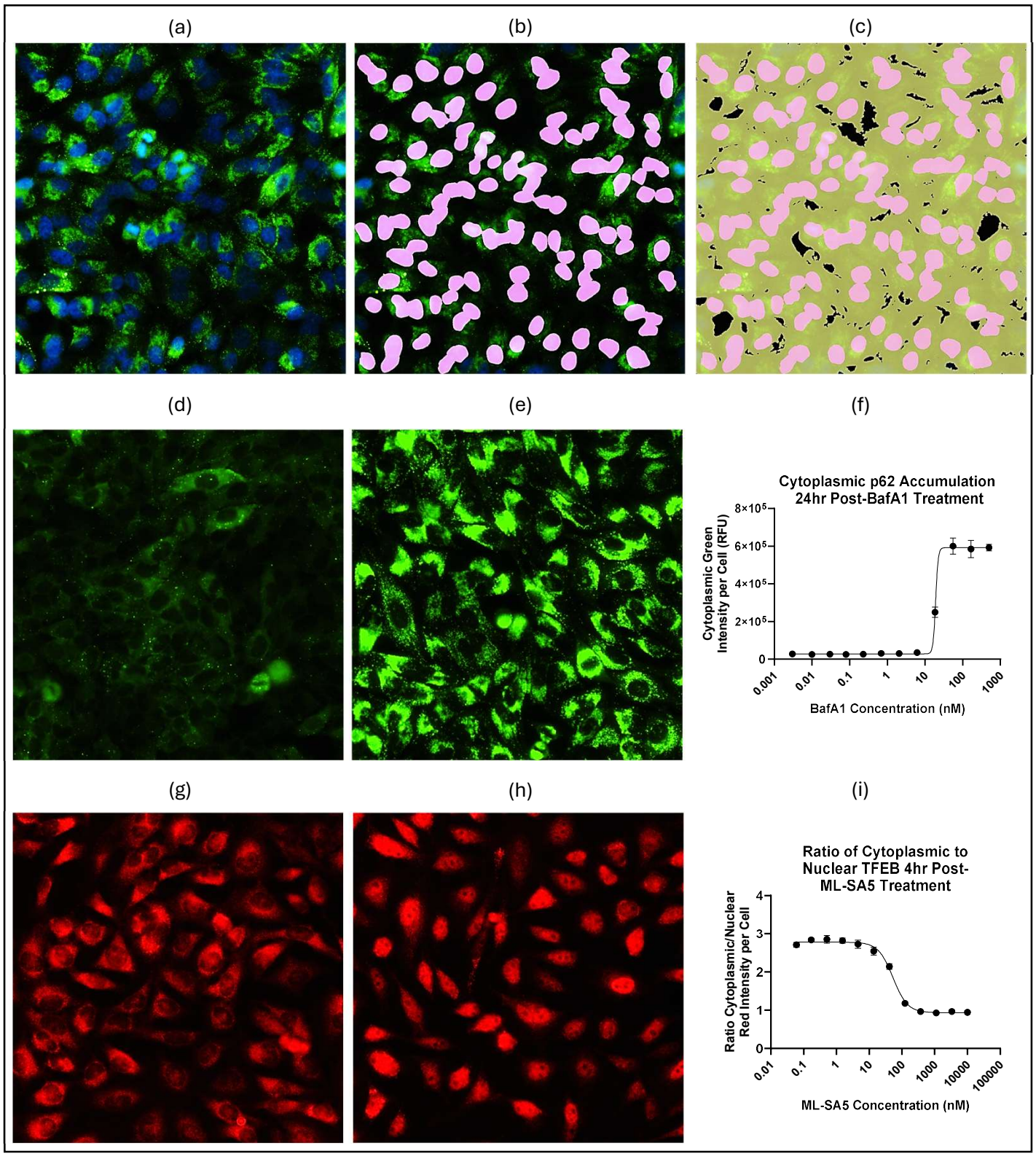

Figure 1. Masking and representative images. (a) Representative HCI image (40X) of nuclei (blue) and p62 (green) staining. (b) Representative images (40X) of nuclear mask (magenta), identified by Hoechst nuclear stain and (c) cell boundary mask (yellow), identified by AF488 p62 staining. (d) Representative image (40X) taken 24-hours after a 0.01nM treatment with BafA1. (e) Representative image (40X) taken 24-hours after a 133nM treatment with BafA1. (f) Graph reporting the increase in green (p62) cytoplasmic intensity/cell following multi-dose treatment of BafA1 (0.08nM-500nM). (g) Representative image (40X) taken 4-hours after a 0.5nM treatment with ML-SA5. (h) Representative image (40X) taken 4-hours after a 10,000nM treatment with ML-SA5. (i) Graph reporting the increase in TFEB nuclear localization following multi-dose treatment of ML-SA5 (10uM-0.17nM).

SUMMARY

BafA1 treatment resulted in aggregation and accumulation of p62 in the cytoplasm, suggesting that lysosomal function was disrupted (images D, E, and F).

ML-SA5 treatment resulted in translocation of TFEB from the cytoplasm to the nucleus in a dose-dependent manner, suggesting that TRPML1 agonism by ML-SA5 resulted in TFEB dephosphorylation (images G, H and I).

EXPLORE BEANTOWN BIOTECH CAPABILITIES

Beantown Biotech is a Boston's local Contract Research Organization (CRO) that provides a comprehensive set of services designed to accelerate the discovery and development of new drugs. Our expertise in discovery biology includes assay development and screening utilizing a wide variety of biophysical, biochemical and cell-based assays.

Leveraging diverse techniques and state-of-the-art instruments, Beantown Biotech is a one-stop location for your assays. Our team of scientists is highly experienced across assay formats and instrumentation, including:

- **Flow cytometry**
  - *Beckman Coulter CytoFLEX S*
- **Meso Scale Discovery (MSD) immunoassays**
  - *MesoScale MESO Quickplex® SQ 120MM MSD microplate reader*
- **High content imaging (HCI)**
  - *PerkinElmer Operetta®*
  - *GE IN Cell Analyzer 6500 HS*
- **Live cell imaging**
  - *Sartorius Incucyte® S3*
- **Real-time PCR**
  - *Roche LightCycler® 480 II*
- **Digital PCR**
  - *QIAGEN QIAcuity One*

And more

LEARN MORE

To learn more, visit our website at https://www.beantownbiotech.com or contact us at info@beantownbiotech.com or 1-508-975-4753.

To request more information or a quote, please fill out our contact form at https://www.beantownbiotech.com/contact.

BTBT
BEANTOWN BIOTECH

REFERENCES

1. InvivoGen, Bafilomycin A1 – Endosomal Acidification Inhibitor, InvitroFit Autophagy modulator, https://www.invivogen.com/bafilomycin-a1.
2. Hilman, L., February 8, 2023, ML-SA5 is a Potent TRPML1 Cation Channel Agonist, Network of Cancer Research, https://www.cancer-research-network.com/2023/02/08/ml-sa5-is-a-potent-trpml1-cation-channel-agonist/