YOKOGAWA

Application Note

Autophagy Analysis

Introduction

Autophagy is the mechanism of the cell that disassembles unnecessary proteins and organelles to recycle or metabolize them. When placed in certain physiological conditions, the cells produce phagophore to cover unnecessary components and form autophagosome. The autophagosome fuses with lysosome (production of autolysosome), and acid hydrolase from lysosome degrades the unnecessary components. Autophagy has been confirmed to be related to cancer and neurodegenerative diseases such as Alzheimer and Parkinson's disease, and as possessing a physiological function in aging.
The following are the results of experiments using the CQ1 for imaging and the high content analysis software CellPathfinder for analysis. In this experiment, DALGreen-Autophagy Detection (Donjindo Molecular Technologies, Inc.)*1 was used; it penetrates cell membranes and is drawn into autophagosome along with unnecessary components, fusing with lysosome and then increasing fluorescence in the acid environment in autolysosome.

Experiment Procedure for Autophagy Analysis through Time-lapse Observation

- HeLa cells were cultured in 96-well plates (Greiner #655087) for 24 hours.
- DALGreen – Autophagy Detection was added according to the attached protocol and incubated for 30 minutes.
- The cells were washed in the cultured medium, and then medium were changed to normal medium, autophagy inducer medium (not containing amino acid) and autophagy inhibition medium (Bafilomycin added to the inducer medium at the final concentration of 100nM). Time-lapse imaging using the CQ1 was implemented every 30 min, for 6 hours (object lens magnification of 20x, 4 fields, 6 Z-slices per well), and fluorescence images processed with DALGreen (Ex:405nm/Em:500-550nm) and bright field images were captured.
- Contrast-enhanced Bright Field (CE Bright Field) images*2 were created from the bright field images using the CellPathfinder, and the cells were counted. Granules produced through autophagy were analyzed in the fluorescence images.

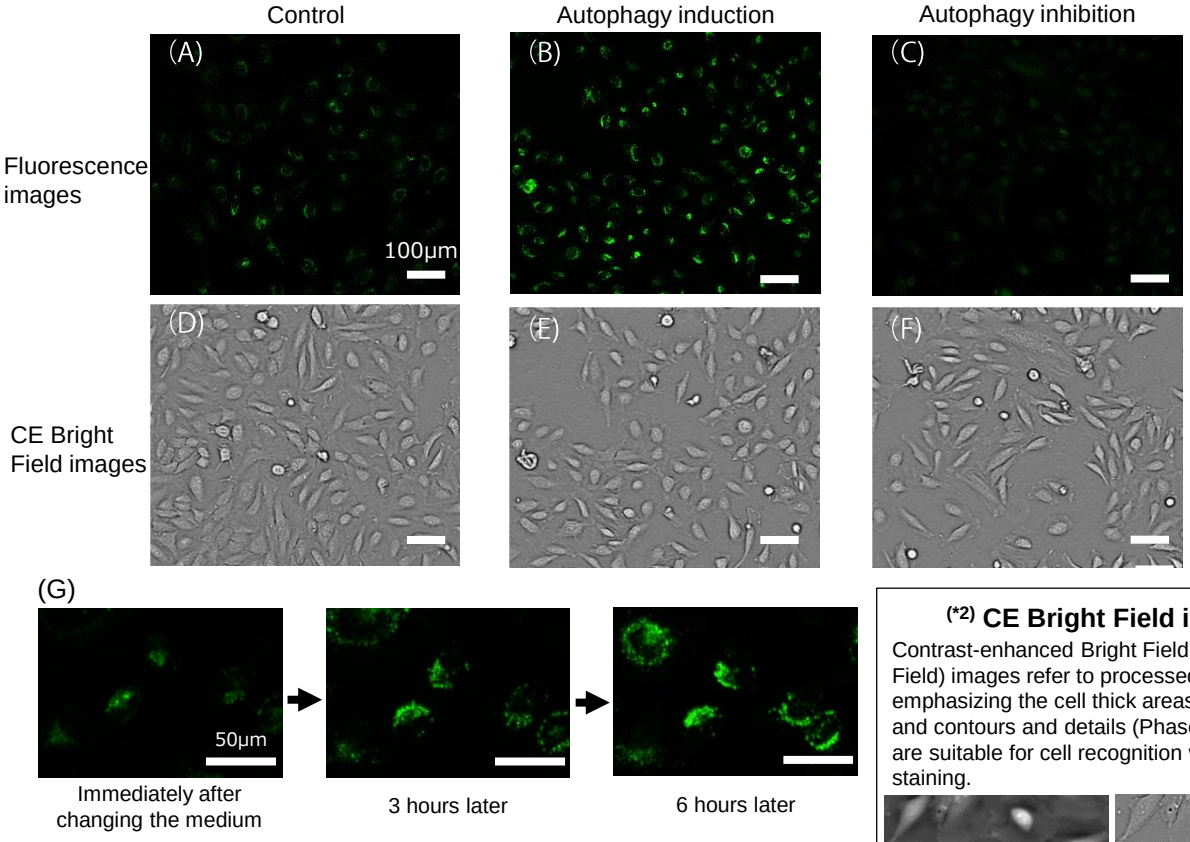
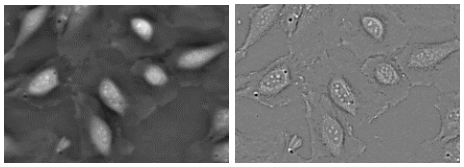


Fig. 1: Autophagy detection
Fluorescence images of the control (A), autophagy induction (B) and autophagy inhibition (C) 6 hours after changing the medium
(D) (E) (F): CE Bright Field images of (A), (B) and (C) respectively (Scale bar: 100 μm)
(G): Images (from left) immediately after changing the medium, 3 hours later and 6 hours later (Scale bar: 50 μm).

(*2) CE Bright Field images

Contrast-enhanced Bright Field (CE Bright Field) images refer to processed images emphasizing the cell thick areas (Fluor type) and contours and details (Phase type), which are suitable for cell recognition without staining.



Fluor type (Emphasizing thick areas)

Phase type (Emphasizing contours and details)

*1 Reference: Iwashita et al., Small fluorescent molecules for monitoring autophagic flux, FEBS Lett,2018,592(4),559.

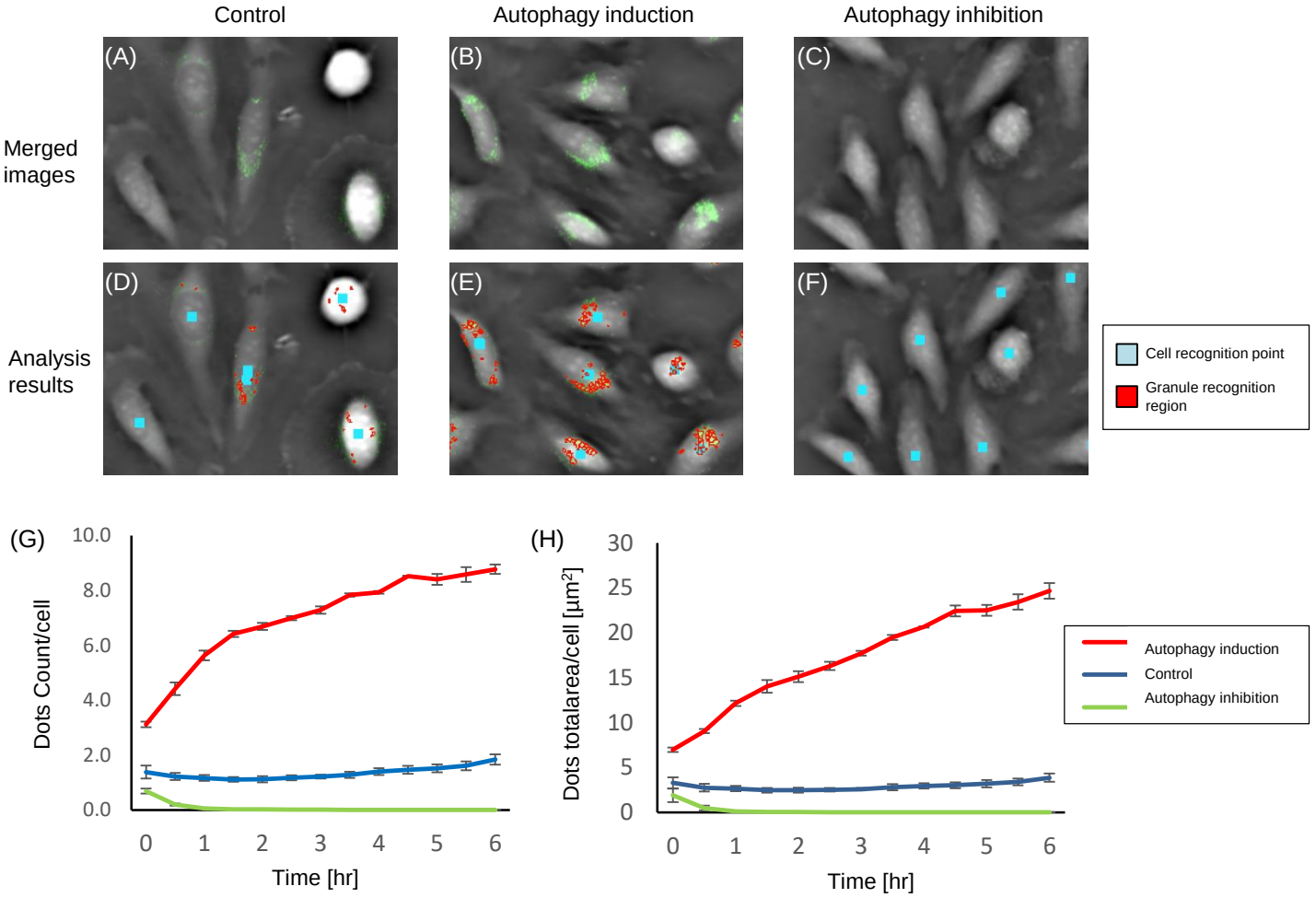


Fig. 2: Autophagy analysis result
Merged images of the control (A), autophagy induction (B) and autophagy inhibition (C) 6 hours after changing the medium.
(D) (E) (F): Analyzed images of (A), (B) and (C) respectively (Blue points: centers of recognized cells; Red: recognized regions of autophagy granules)
(G) (H): Change of count and total area of granules per cell over time. Error bars indicate SE (n=3). (Blue: control, Red: autophagy induction, Green: autophagy inhibition)
The count and total area of granules increased over time only in the autophagy induction.

Results and Discussion

It was confirmed that live cell autophagy can be easily observed using the CQ1 and DALGreen-Autophagy Detection. Also, autophagy was induced in the media not containing amino acid and inhibited by adding Bafilomycin. The CQ1 allows for the observation of changes with time while maintaining the culture environment through the use of its stage heater for the control of temperature and humidity, as well as the concentrations of CO_2 and O_2 through the combined use of a gas mixer.

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