Killing/ADCC Assay by NK cells

Introduction

In recent years, NK cells, which play a major role in eliminating tumors and virus-infected cells and in antibody-dependent cell-mediated cytotoxicity (ADCC), have attracted attention. Killing assay and ADCC assay, which measure the cytotoxic activity and ADCC activity of NK cells, use fluorescent staining reagents, but there are problems such as increased costs and cell damage. In this experiment, we used Cell3iMager NX and deep learning functions to perform label-free Killing/ADCC assay by NK cells against breast cancer cells.

Materials&Methods

Product Used

Cell3iMager NX

Samples & Reagents

Primary NK cell (Biotherapy Institute of Japan)
MCF7 cell line (RIKEN)
BT474 cell line (ATCC)
NK cell dedicated medium (Biotherapy Institute of Japan)
DMEM (Nacalai tesque)
RPMI (Nacalai tesque)
FBS (Biosera)
96 well plate Flat bottom (SUMILON)
96 well plate U-shape bottom (SUMILON)
CellTrace Far Red (Thermo)
Trastuzumab (anti-HER-2-ab)

Methods

HER-2-negative MCF7 cells and HER-2-positive BT474 cells fluorescently stained with CellTrace Far Red. The cells were cultured in a 2D/3D culture method for 72 hours, then co-cultured with NK cells and Trastuzumab was added. After 96 hours, Killing/ADCC assay were performed by Cell3iMager NX. Next, using the deep learning function, we labeled the cancer cell area in the bright-field image and created a teacher image. By referring to the fluorescence image when labeling the brightfield image, it is easier to distinguish between cancer cells and NK cells in the bright-field image(Fig. 1).

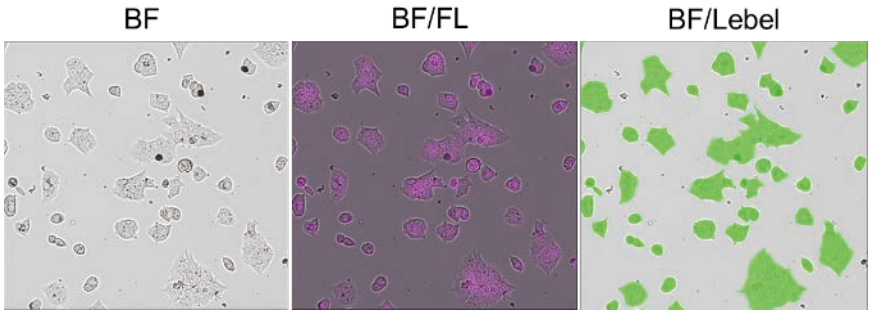

Fig. 1 : Created teacher image

A deep learning model was created by learning for 3 hours using the teacher dataset (bright-field image and teacher image). By using the model to an unknown image, the cancer cells in the bright-field image were segmented and measured the feature values.

Results

2D Killing/ADCC Assay

Segmentation of the cancer cell area in the bright-field image and quantification of the area revealed that the area decreased depending on the number of NK cells. We found that Trastuzumab had no effect on HER-2-negative MCF7 cells (Fig. 2, Fig. 3), but decreased the area of HER-2-positive BT474 cells. It suggests that Trastuzumab enhanced NK cell cytotoxicity (Fig. 4, Fig. 5).

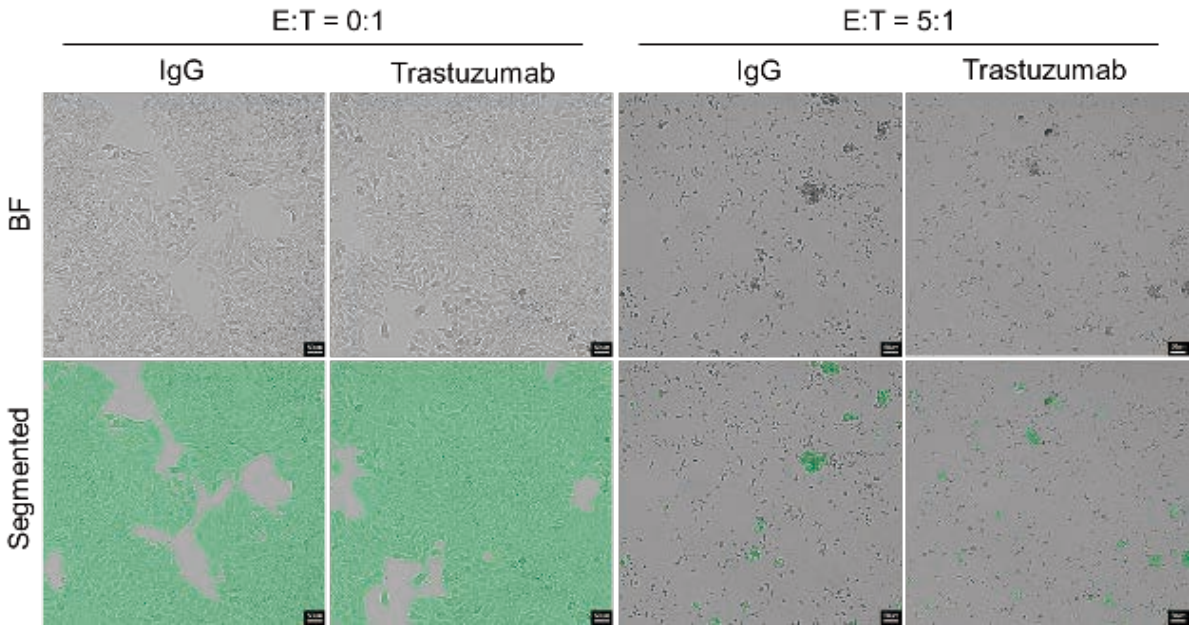

Fig. 2 : Images of MCF7/NK cells (2D)

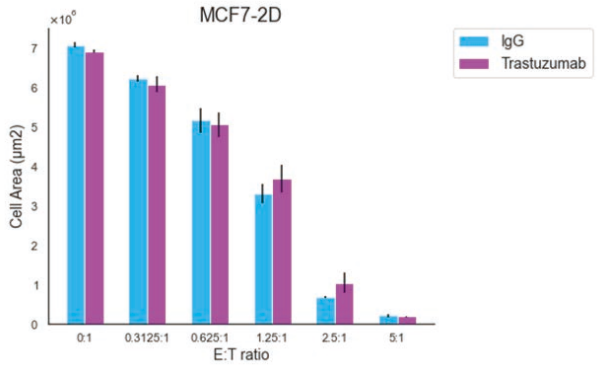

Fig. 3 : Result of MCF7/NK cells (2D)

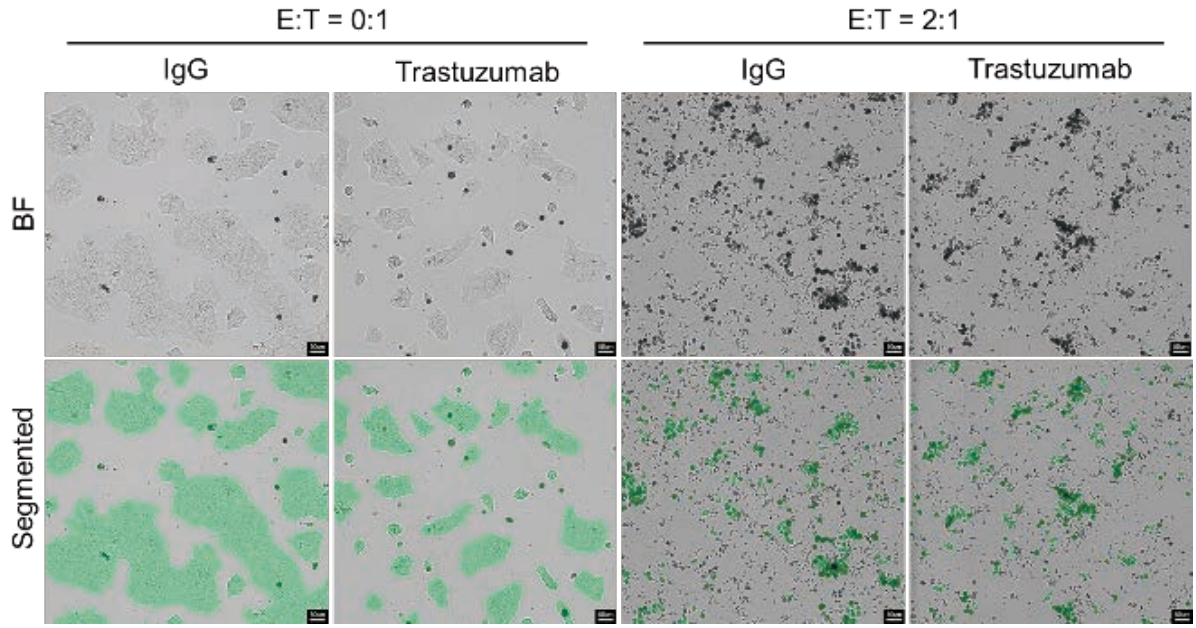

Fig. 4 : Images of BT474/NK cells (2D)

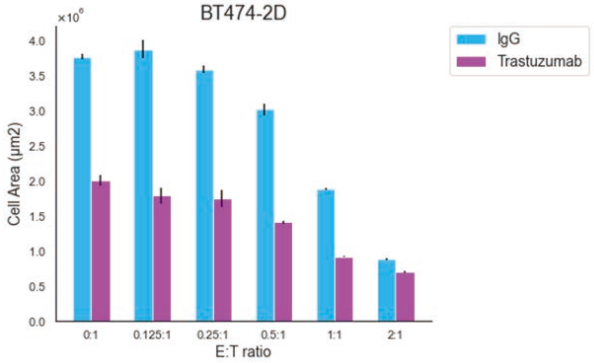

Fig. 5 : Result of BT474/NK cells (2D)

3D Killing/ADCC Assay

Segmentation of the cancer spheroid area in the bright-field image and quantification of the area revealed that the area decreased depending on the number of NK cells. We found that Trastuzumab had no effect on HER-2-negative MCF7 cells (Fig. 6, Fig. 7), but decreased the area of HER-2-positive BT474 cells. It suggests that Trastuzumab enhanced NK cell cytotoxicity (Fig. 8, Fig. 9).

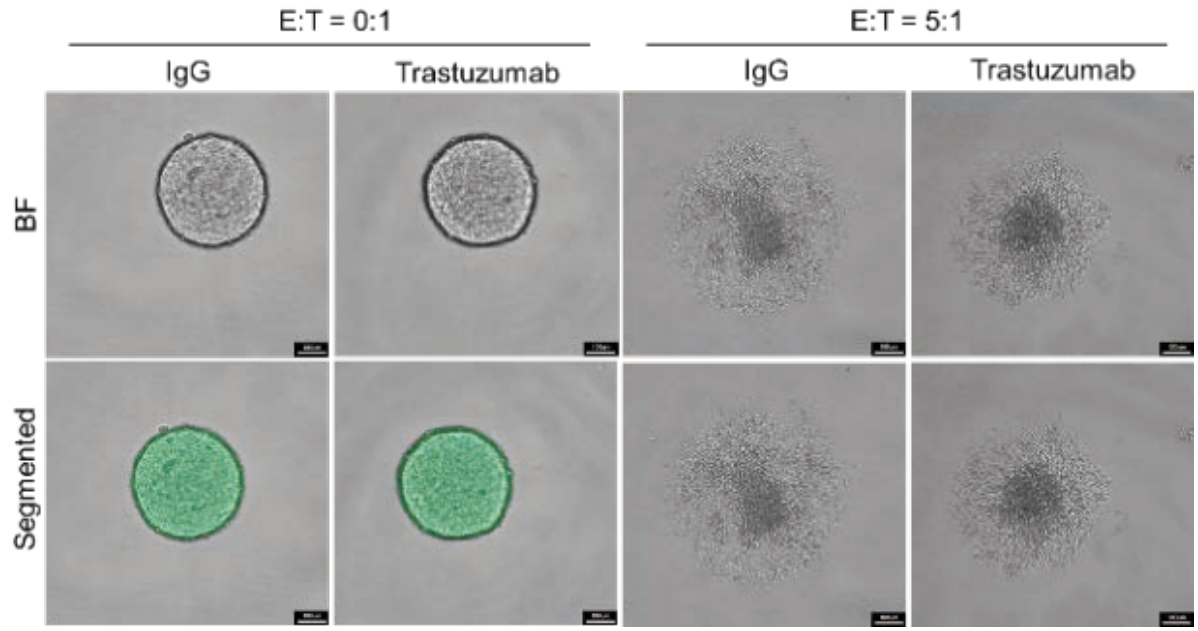

Fig. 6 : Images of MCF7/NK cells (3D)

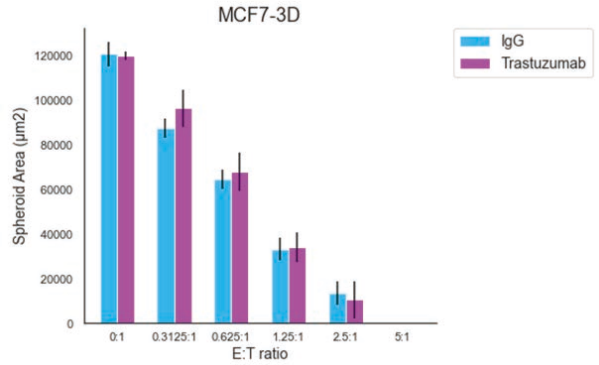

Fig. 7 : Result of MCF7/NK cells (3D)

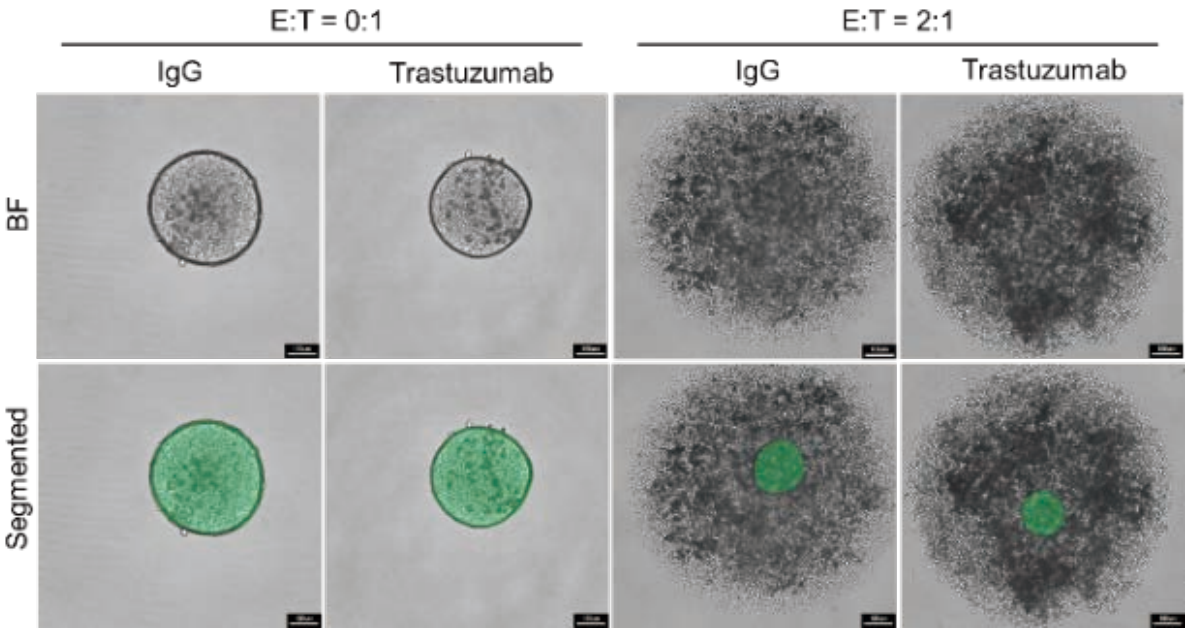

Fig. 8 : Images of BT474/NK cells (3D)

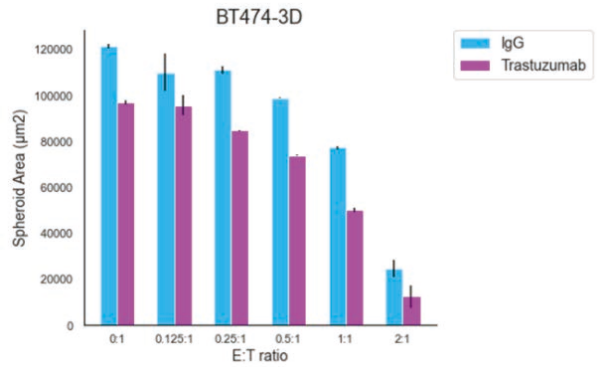

Fig. 9 : Images of BT474/NK cells (3D)

Conclusion

By using Cell3iMager NX, bright-field/fluorescence imaging of 2D/3D cultured cancer cells and NK cells is possible. In addition, by using deep learning, it is possible to segment only cancer cells in bright-field images, so even biotechnology researchers who are not familiar with image processing and machine learning can easily perform label-free Killing/ADCC assay analysis.

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