Quantification of Natural Killer Cell–Mediated Cytotoxicity using Celigo Imaging Cytometry

ABSTRACT

Cytotoxicity assays play a central role in studying the function of immune effector cells such as cytotoxic T lymphocytes (CTL) and natural killer (NK) cells. Traditionally, cytotoxicity assays have been performed using Chromium (⁶⁷Cr) and Calcein release assays. The assays involve labeling tumor cells (target) with radionuclide or fluorescent dyes, when the target cells are subjected to cytolysis by CTLs or NK cells (effector), they release the entrapped labels into the media upon lysis. The amount of labels in the media is measured to determine the level of cytotoxicity the effectors have induced. These traditional methods may generate inconsistent results due to low sensitivity caused by poor labeling efficiency and high spontaneous release of the reagents. In this work, we demonstrate a novel cytotoxicity assay using the Celigo imaging cytometry method. Utilizing imaging cytometry, direct cell counting of live fluorescent target cells can be performed, which is a direct method for assessment of cytotoxicity. Human NK cells from one healthy donor were used as effectors, and K562 (suspension) and IMR32 (adherent) were used as the target cells. Both target cells were first stained with Calcein AM, and seeded at 10,000 cells/well in a standard 96-well microplate. The donor NK cells were then added to each well at Effector-to-Target (E:T) ratios 10:1, 5:1, 3:1, 1.5:1, 1:3:1, 0.3:1, 0.6:1, and 0.3:1/5. The 96 well plate was then scanned and analyzed using Celigo imaging cytometer at t = 1, 2, 3, 4 h to measure the % lysis of target cells. The results showed increasing % lysis as incubation time and E:T ratio increased. The proposed Celigo imaging cytometry is an accurate and simple method for direct quantification of cytotoxicity, which can be an attractive method for both academic and clinical research.

1. ABSTRACT

2. CELIGO IMAGING CYTOMETRY FOR DIRECT CELL COUNTING ADCC ASSAY

3. TRADITIONAL CELL-MEDIATED CYTOTOXICITY DETECTION METHODS

4. NATURAL KILLER CELL-MEDIATED CYTOTOXICITY DETECTION METHOD

5. CELIGO IMAGING CYTOMETRY EXPERIMENTAL PROTOCOL

6. E:T RATIO AND TIME DEPENDENT CYTOTOXICITY FLUORESCENT IMAGES OF IMR32

7. E:T RATIO AND TIME DEPENDENT CYTOTOXICITY RESULTS OF IMR32

8. E:T RATIO AND TIME DEPENDENT CYTOTOXICITY FLUORESCENT IMAGES OF K562

9. E:T RATIO AND TIME DEPENDENT CYTOTOXICITY RESULTS OF K562

10. SUMMARY AND CONCLUSION