Using Impedance-Based Approaches for Measuring Cell-Mediated Cytotoxicity and Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

Leyna Zhao, Ph.D. ACEA Biosciences Inc. San Diego, California. USA

ACEA Biosciences, Inc.

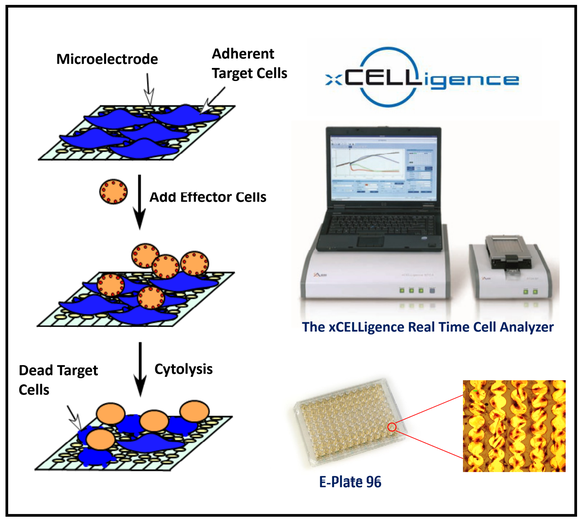
INTRODUCTION

The most common method for measuring cell mediated cytotoxicity is the release assay based on the loss of target cell membrane integrity. Within up to 4 hours following effector cell addition resulting in target cell lysis, either the radioactive release from target cells pre-labeled with Chromium-51 or Indium-111 is measured, or the release of naturally occurring substances, such as lactate dehydrogenase (LDH), into the culture medium is assayed. Release of these substances thus serves as an indirect measure of the extent of cell damage due to effector cell-mediated target cell lysis. Alternative endpoint methods also include flow cytometry, enzyme-linked immunosorbent assay-based granzyme measurement, and morphometric analyses by microscopy.

Here we describe an impedance-based real-time label-free method that can automated capture the kinetics of the cell mediated or antibody-dependent cell mediated cytolysis (ADCC) of the target cancer cells. To determine if cell-mediated cytotoxicity, and specifically ADCC, can be investigated using an impedance-based xCELLigence system, the response of tumor cells (as target cells) to natural killer (NK) cell activity (as effector cells), in the presence or absence of immunoglobulin G isotype-specific antibody, was measured. Importantly, it is shown that the addition of NK cells in suspension, over a monolayer of adherent tumor cells, does not produce impedance changes, because the NK cells do not come in contact with the electronic sensor. However, the secretion of perforins and granzymes by these non-adherent NK cells does activate caspases inducing tumor cell apoptosis. Dysfunctional and dying tumor cells detach from the sensor electrode, reducing the number of viable and adhering cells on the electrode surface.

Overall, our results show that the impedance-base technology is a label-free alternative to the traditional end-point assays. The automated readouts provide direct, sensitive and specific measurement of target cell changes both short-term (hrs) and long-term (days). It allows the easy quantification of the cell-mediated cytotoxicity and evaluation of the potencies for specific antibodies.

METHODS



RESULTS

1. Dynamic Monitoring of NK Cell-mediated Cytolysis

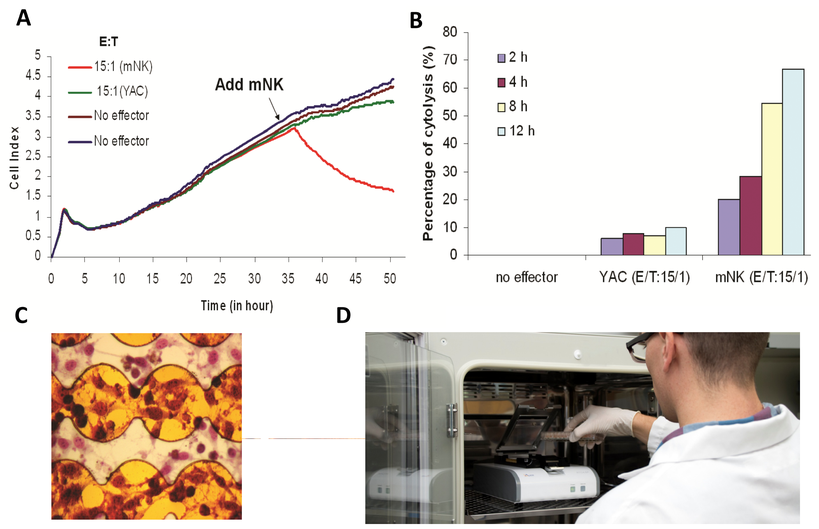


Fig 1. (**A**) Dynamic monitoring of NK cell-mediated cytolysis of NIH 3T3 cells. Mnk cells or YAC (negative control) cells were added to each well in triplicate. (**B**) Time-dependent cytolytic activity of mNK cells. The cytolytic activity at a given time point was calculated and presented as the percentage of cytolysis (**C**) The morphological examination of cytolysis by mNK cells. (D) The xCELLigence RTCA SP system in a conventional tissue culture incubator.

3. Real-time, label-free monitoring of NK92 cell-mediated cytolysis of DU145 cells

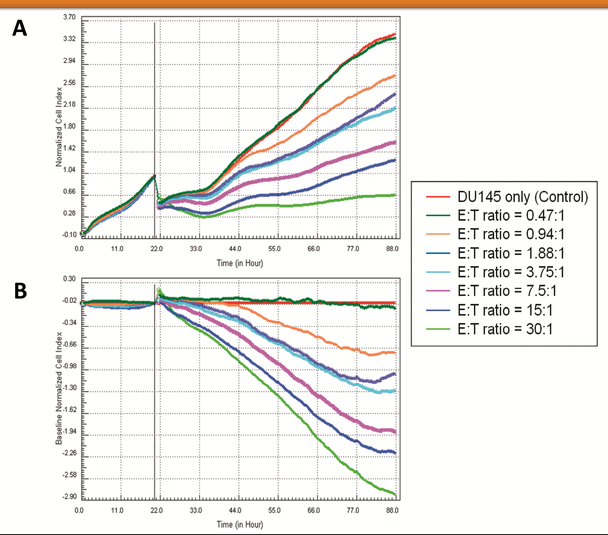


Fig 3. (**A**) This plot shows data normalized to the last time point before NK cell addition. (**B**) Same data as (A), but with non-treated control cells (red) defined as baseline. Plots were generated using RTCA Software 1.1.

4. Effect of anti-IGF-1R Ab on NK cell-mediated cytolysis of DU145 cells (E:T=2.75:1)

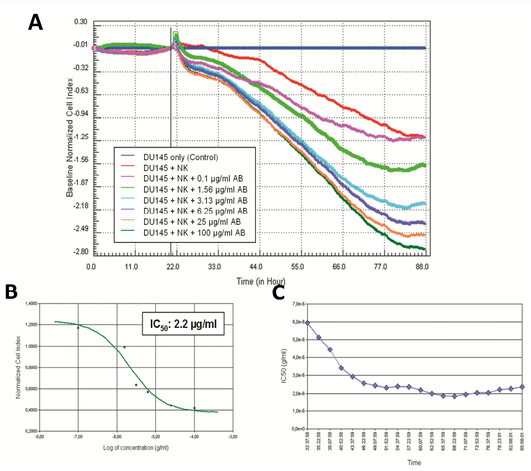


Fig 4. (**A**) Plot of normalized CI values of the entire 88 hours of the experiment. Data are normalized to the last time point before NK cell addition and curves were plotted with control wells (DU145 cells only) set as baseline. (**B**) Calculation of IC50 after 60 hours. (**C**) Calculation of time-dependent IC50 from 9 hours after NK cell addition to the end of the experiment at 88 hours. All plots were generated using the RTCA Software.

2. Label-free and quantitative measure of cytolytic activity of mNK cells and NK92 cells

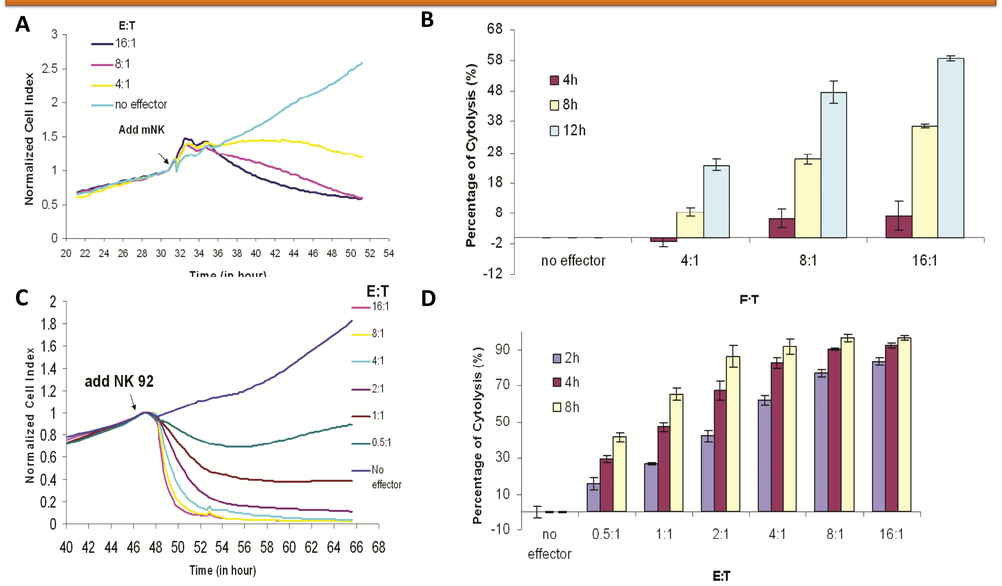


Fig 2. (**A**) Quantitative measurement of cytolytic activity of mNK cells on NIH 3T3 target cells. (**B**) Time-dependent cytolytic activity of mNK cells at different E/T ratios. (**C**) Quantitative measurement of cytolytic activity of NK92 cells on MCF7 target cells. (**D**) Time-dependent cytolytic activity of NK92 cells at different E/T ratios.

5. Label-free assessment of NK cell-mediated cytolysis using a variety of target cell lines

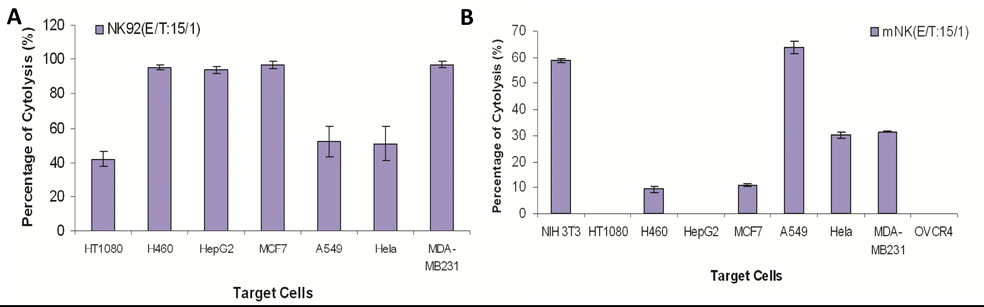


Fig 5. (**A**) The NK92-mediated cytolysis of 7 different cancer lines. The percentage of cytolysis indicated for each line is calculated based on the Cell Index value of individual wells 8 hours after NK92 cells were added; cytolysis reached a maximum at that time. (**B**) The mNK-mediated cytolysis of 9 different cell lines. The percentage of cytolysis indicated for each line is calculated based on the Cell Index values of individual wells 12 hours after mNK cells were added; cytolysis reached a maximum at that time.

SUMMARY

The present study shows the feasibility for using the xCELLigence System to monitor both NK cell-mediated tumor cell cytolysis and antibody dependent cell-mediated cytotoxicity (ADCC) in a label-free, non-invasive manner, corroborating earlier findings by Glamann and Hansen (2006). It was possible to show both the quantitative effect of adding different amounts of NK92 cells, as well as the additive cytolytic effect of introducing an NK92-dependent anti-IGF-1R monoclonal antibody.

The xCELLigence System is thus ideal for directly monitoring ADCC without the need for labeling the target cells or using a chemical reporter. Importantly, the entire cytolytic process can now be measured using impedance electronic sensing to detect both expected and unexpected cellular responses, an impossible task for previously described conventional end-point assay formats.

SELECTED PUBLICATIONS

1. Determining optimal cytotoxic activity of human Her2neu specific CD8 T cells by comparing the Cr51 release assay to the xCELLigence system. Erskine CL, Henle AM, Knutson KL.J Vis Exp. 2012 Aug 8;(66):e3683
2. Real-time profiling of NK cell killing of human astrocytes using xCELLigence technology. Moodley K, Angel CE, Glass M, Graham ES. J Neurosci Methods. 2011 Sep 15;200(2):173-80
3. Unique functional status of natural killer cells in metastatic stage IV melanoma patients and its modulation by chemotherapy. Fregni G, Perier A, Pittari G, Jacobelli S, Sastre X, Gervois N, Allard M, Bercovici N, Avril MF, Caignard A. Clin Cancer Res. 2011; 17(9); 2628–37.