Immune Cell Killing Assay for Measuring the Effects of Immunomodulating Agents in Lung Cancer Cells *In Vitro*

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Introduction

Cancer immunotherapy is one of the most promising new approaches in cancer drug development. Direct effects of cancer therapeutics on immune cell activity can be challenging to determine on system level *in vivo*. Therefore, it is important to develop and discover novel ways to study the effects of immune cells on cancer cells using also *in vitro* assays.

Our aim was to validate an *in vitro* immune cell killing assay where activated peripheral blood mononuclear cells (PBMCs) target lung cancer cells and induce apoptosis.

Materials and Methods

A549 human lung carcinoma cells (ATCC) were used as target cells in the co-culture and were plated into 96-well plates. After 24 hours, human PBMCs (Lonza) were added to the culture as effector cells and activated with anti-CD3 and IL-2. Apoptosis was detected using IncuCyte Annexin V reagent, which was added to the cells at the same time with immune cell activators. Cell proliferation was monitored with a live cell imager IncuCyte S3 (Sartorius) for three to four days before terminating the cultures. Cell viability was determined by CellTiter Glo (Promega) at the end of the study.

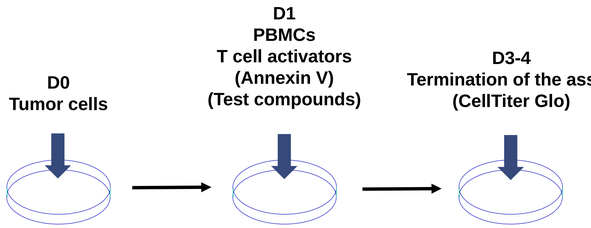

Figure 1. Timeline of the study. Tumor cells are seeded in the beginning of the assay. One day later, the PBMCs and their activators, anti-CD3 and IL-2, are added to the cultures. Annexin V and possible test compounds are added at the same time. The cells are followed by IncuCyte S3 live cell imager for 3-4 days. Cell viability is measured at the end of the study using CellTiter Glo cell viability assay.

Results

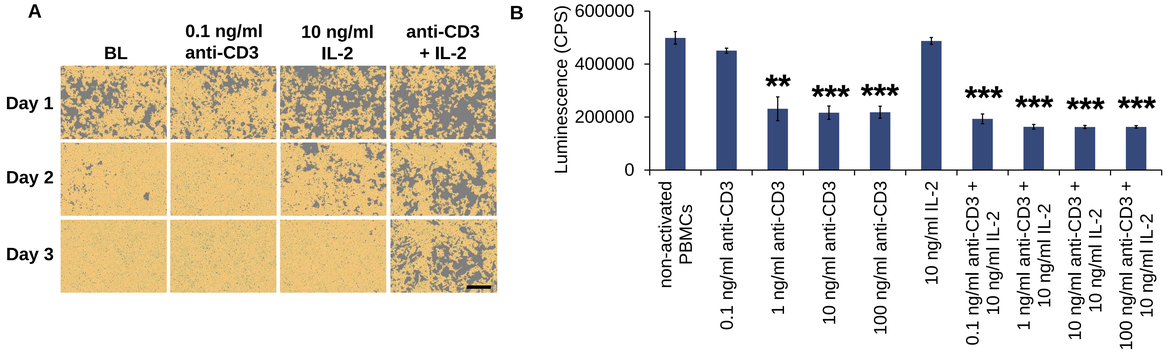

Figure 2. Confluence of A549 cells in the presence of PBMCs and T cell activators anti-CD3 (0.1-100 ng/ml) and IL-2 (10 ng/ml) with a target-to-effector ratio of 1:5. A) Cells were imaged with two hour intervals by IncuCyte S3. Representative images one, two and three days after treatment initiation are shown with IncuCyte confluence mask (yellow). BL = co-culture of A549 and PBMCs without activators. Scale bar = 400 µm. B) Cell viability of co-culture was measured at the end of the study using CellTiter Glo cell viability assay.

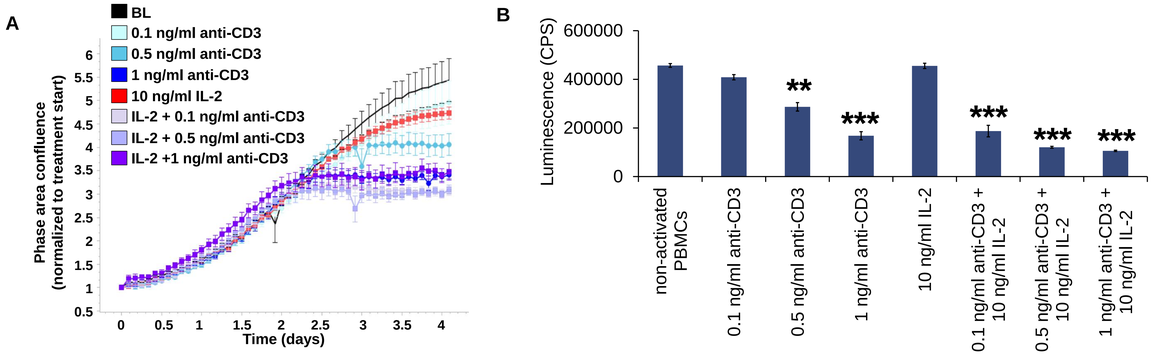

Figure 3. Confluence of A549 cells in the presence of PBMCs and T cell activators anti-CD3 (0.1-1 ng/ml) and IL-2 (10 ng/ml) with a target-to-effector ratio of 1:5. A) Cells were imaged with two hour intervals by IncuCyte S3. The data are from three wells per treatment and two images per well (mean± SEM). 0 h = seeding of tumor cells. BL = co-culture of A549 and PBMCs without activators. B) Cell viability of co-culture was measured at the end of the study using CellTiter Glo cell viability assay.

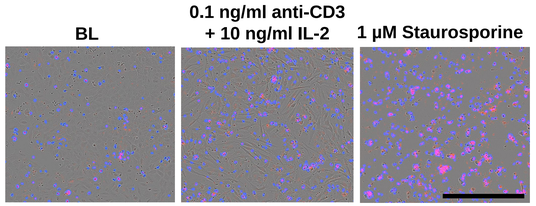

Figure 4. Apoptosis of A549 cells in the presence of PBMCs and T cell activators anti-CD3 (1 ng/ml) and IL-2 (10 ng/ml) or Staurosporine (1 µM) with a target-to-effector ratio of 1:5. Cells were imaged by IncuCyte S3 for three days after adding treatments. Apoptosis was measured using IncuCyte Annexin V Red Reagent, which was added to cultures at the same time with immune cell activators. Representative images at the end of the assay are shown with IncuCyte apoptosis mask (blue). BL = co-culture of A549 and PBMCs without activators. Scale bar = 400 µm.

Conclusions

Treatment of cells with anti-CD3 was able to activate immune cells alone but the effects were observed with lower test concentrations in combination with IL-2.

Therefore, IL-2 potentiates the effect of anti-CD3 on immune cell activation and decreases cancer cell viability in co-culture.

The immune cell killing assay based on live cell imaging can be used for studying the effects of immunomodulating agents on cancer cell viability and to evaluate the activity of immune cells in the presence of therapeutics.

Acknowledgements

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