High-throughput immune complex analysis method for CAR T cell-mediated cytotoxicity using the Celigo Image Cytometer

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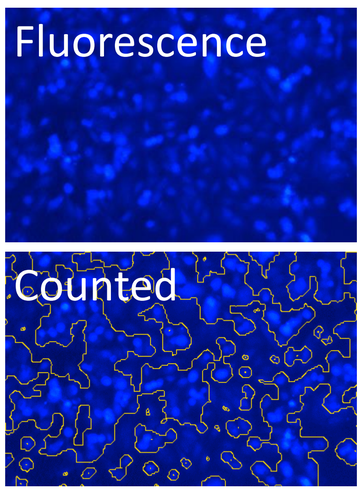
1. ABSTRACT

Cancer immunotherapy has been gaining momentum in the field of cancer research. Specifically, Chimeric Antigen Receptor (CAR) T cell technology have introduced new methods to combat cancer. Direct cell-mediated cytotoxicity assays are required to assess the killing capability of the engineered CAR T cells. Traditionally, these assays are conducted by measuring the amount of released Chromium, calcein AM, or LDH molecules after the target cancer cells are killed with CAR T cells. These methods require a large amount of target cells which may not be ideal when working with donor primary samples. Additionally, they cannot specifically analyze the immune complexes formed during CAR T cell killing. Immune complex formation is a known event that may reveal information for effector cell migration. Recent advancement in imaging technologies have developed novel methods for assessing these immune complexes. In this work, we demonstrated an immune complex analysis method using the Celigo Image Cytometer to determine CAR T cell activity during co-culture with cancer cells. First, different target cancer cells (HEK293T, MDA-MB-231, MDA-MB-468, HCC38, and skrc59) are stained with ViaStain™ Tracer Blue dye, seeded in a 96-well plate, and incubated overnight. Next, untransduced T cells and G36-CAR T cells are added to the wells at 20:1 effector-to-target (E:T) ratios and co-cultured for 24 hours. Finally, the plate is scanned and the immune complexes were analyzed by confluence measurement in blue channel and compared to the untransduced T cells. Image cytometry analysis evaluated CAR T cell activities for all of tested target and effector cell combinations. Utilizing an image cytometry platform can visually confirm interactions between effector and target cells, thus making results highly accurate and robust. Unlike the traditional release assays, the ability to analyze the immune complexes formed during co-culture assays can provide additional important functional information of the effector cells, such as migration and clustering capabilities.

2. CELIGO IMAGING CYTOMETRY FOR CAR T CELL IMMUNE COMPLEX



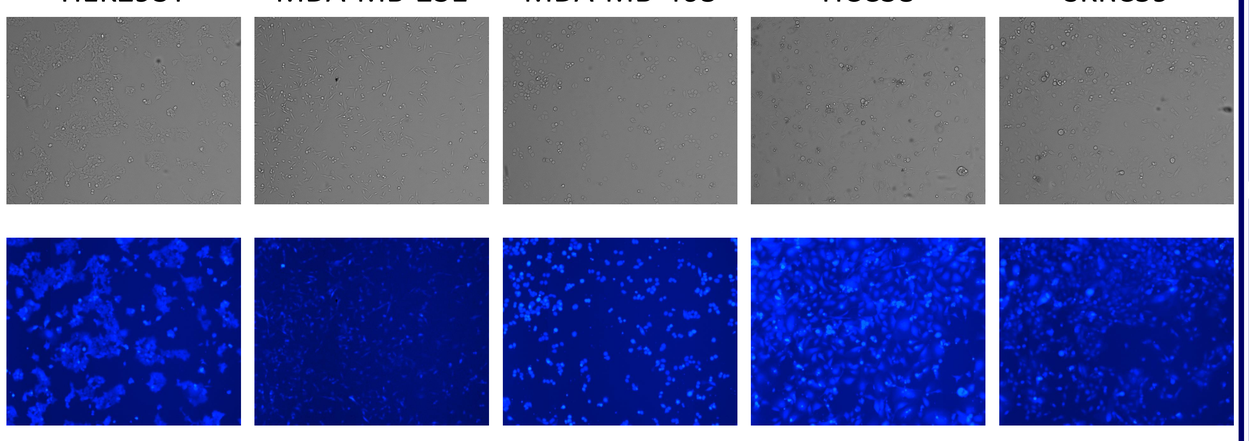
1. Celigo Imaging Cytometer is a plate-based cytometer that can scan the entire well of standard microplates and capture bright-field and fluorescent images
2. The captured images are analyzed with the Celigo software to measure size, morphology, cell count, confluence, and fluorescent intensity
3. The measured parameters are used to generate cell proliferation kinetic data, GFP/RFP expression, tumor spheroid size change, DNA cell cycle analysis, apoptosis, and ADCC cytotoxicity results

3. CAR T CELL-MEDIATED CYTOTOXICITY ASSAY PROTOCOL

	1	2	3	4	5	6	7	8	9	10	11	12
A	HEK293T			HEK293T + UN			HEK293T + G36					
B	MDA-MB-231			MDA-MB-231 + UN			MDA-MB-231 + G36					
C	MDA-MB-468			MDA-MB-468 + UN			MDA-MB-468 + G36					
D	HCC38			HCC38 + UN			HCC38 + G36					
E	SKRC59			SKRC59 + UN			SKRC59 + G36					
F				UN			G36					
G	HEK293T		MDA-MB-231	MDA-MB-468		HCC38			SKRC59			
H												

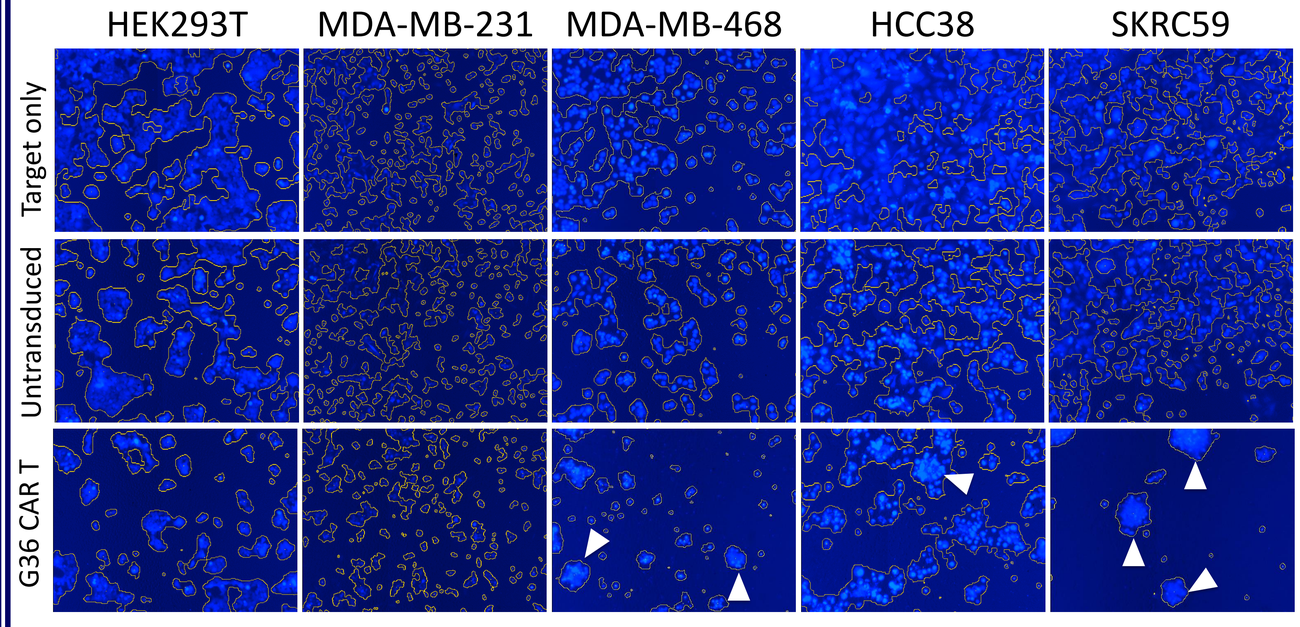
Seed Blue Tracer dye stained cancer cells, incubate overnight → Add in untransduced T cells and G36 CAR T cells → Allow CAR T cell-mediated cytotoxicity to occur over 24 hours → Use the Celigo to perform immune complex analysis

4. BRIGHT FIELD AND FLUORESCENT IMAGES OF TARGET CANCER CELLS

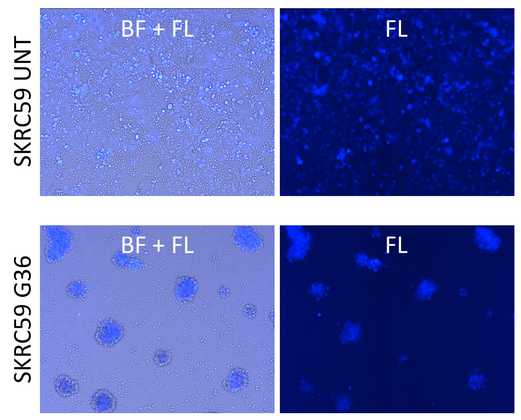


- Different cancer cells show different morphology growing on the surface of the well
- The cancer cells are stained brightly with the Blue Tracer dye

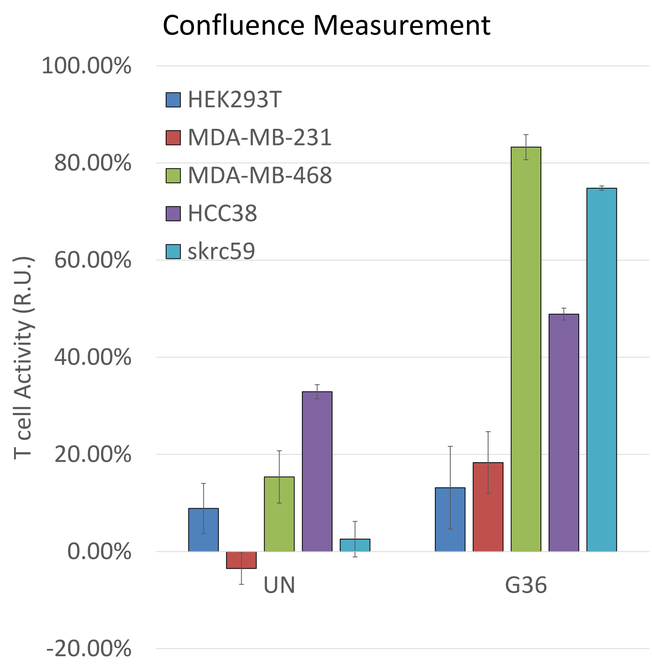
5. CAR T CELL-MEDIATED CYTOTOXICITY AND IMMUNE COMPLEX FORMATION



- Little to no changes in target cancer cells (well confluence or immune complex formation) were observed when co-cultured with untransduced T cells
- G36 CAR T cells co-culture with HEK293T and MDA-MB-231, displayed minimal changes in target cell confluence and no visible formation of immune complexes
- G36 CAR T cells co-cultured with positive control target cell line SKRC59 (Suarez *et al*. 2016) displayed robust target cell killing and formation of immune complexes (dense target cell/T-cell clusters, white arrow)
- G36 CAR T cells co-cultured with MDA-MB-468, HCC38, displayed varying levels of target cell killing with some immune complexes formed (white arrow)
- It showed CAR T cell migration potentially inducing cytotoxic killing of target cancer cells



6. CAR T CELL ACTIVITY EVALUATION FROM IMMUNE COMPLEXES



- The change in target cell confluence % is calculated from time 0 and 24 hours
- Next, the change in confluence % is normalized against the negative control without T cells
  - Higher T cell activities values indicate more formation of immune complexes
- The G36 CAR-T cells showed the greatest T-cell activity against SKRC59 and MDA-MB-468 cell lines
- Both untransduced T cells and G36 CAR T cells had some T cell activity against HCC38 cell line
- Minimal T cell activity was measured for untransduced or G36 CAR T cells on HEK293T and MDA-MB-231 cell lines

7. SUMMARY AND CONCLUSION

- The Celigo was able to rapidly image and analyze the target cancer cells stained with blue tracer dye after 24 hours incubation
- Different cancer cells co-cultured with antigen-specific CAR T cells showed varying levels of CAR T cell activities and the formation of immune complexes
- The formation of immune complexes did not ultimately indicate cytotoxicity but shows migration of immune cells around the target cells, which may potentially induce cytotoxicity of the target cells