Medical Xpress

# Researchers develop innovative and flexible method to study immune cell capabilities

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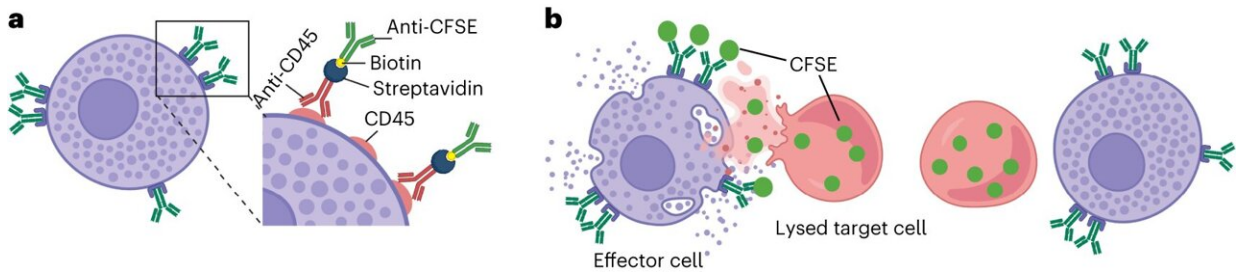


Schematic of PAINTKiller assay. a, Preparation of affinity matrix on effector cells. Effector cells were first labeled with anti-CD45 conjugated with streptavidin, followed by biotinylated anti-CFSE antibody. b, Target cells were labeled with intracellular fluorophore (CFSE). Co-culture of effector cells and target cells results in killing of target cells. Upon lysis, CFSE is released from target cells and is captured by anti-CFSE antibodies on effector cells. Illustrations were created with BioRender.com. Credit: *Nature Biomedical Engineering* (2023). DOI: 10.1038/s41551-023-01089-z

Our body's immune system is a complex network of organs, cells, and proteins that work in synchrony to protect our bodies against infections caused by pathogens and fight against disease-causing changes in our body, such as the emergence of cancer cells. Cell-mediated cytotoxicity is one of such defense mechanisms carried out by the immune system that fights against foreign cells.

Deep analysis of the various players involved in cell-mediated cytotoxicity can provide insights into the normal processes during good health and the defense mechanisms during an autoimmune disorder. However, current methods of analysis are unable to accurately describe the intricacies of cell-mediated cytotoxicity as these methods adopt an indirect approach.

Assistant Professor Cheow Lih Feng from the NUS Institute for Health Innovation & Technology, together with his team members Dr. Luah Yen Hoon and Dr. Wu Tongjin, have devised a simple and innovative way to directly identify and sort the immune cells involved in cell-mediated cytotoxicity.

"The novel methodology proposed by my team is highly selective in identifying the killer cells capable of efficiently eliminating a target cell. This stands in contrast to other cell-mediated cytotoxicity characterization techniques, which are labor-intensive, time-consuming and less selective," said Cheow, who is also from the Department of Biomedical Engineering under the NUS College of Design and Engineering.

This new method was published in *Nature Biomedical Engineering* on 31 August 2023.

## Painting killer cells green

During cell-mediated cytotoxicity, some immune cells can destroy foreign cells through cell lysis by releasing proteins that trigger a cascade of cellular processes that destroy foreign cells. These immune cells that have exhibited cell-killing behavior are referred to as killer cells. Once these foreign cells are killed or lysed, they will spill their intracellular contents to the surrounding area.

Building upon this concept, the NUS team proposed a way to "paint" the surface of the killer cells responsible for destroying these foreign cells. The researchers named this new method PAINTKiller (for "proximity affinity intracellular transfer identification of killer cells").

The NUS researchers used an intracellular staining dye known as carboxyfluorescein succinimidyl ester (CFSE) to stain the foreign cells. This non-toxic dye can enter and be retained within cells. The team then modified the surface of immune cells so that they would have this receptor to capture the CFSE dye when it is released by the foreign cells during cell lysis, allowing them to identify the killer cells that are responsible.

## Sorting killer cells for immunotherapy applications

Following the successful design of the PAINTKiller method, Cheow and his team conducted an experiment to investigate the possibility of using PAINTKiller to sort the subtypes of killer cells involved in cell-mediated cytotoxicity. The team demonstrated that killer cells that were labeled with the CFSE dye displayed better performance in killing foreign cells as compared to killer cells that were not labeled.

Using the CFSE dye as an identifier, the NUS researchers were able to sort and extract the subtypes of killer cells and grow them separately. They also found that immune cells that were labeled by CFSE using the PAINTKiller method had better killing capacity and remained potent even after 12 days.

These promising results suggest that PAINTKiller could provide a potential strategy for producing higher-quality cell-based immunotherapies.

# Expanding the use of PAINTKiller in analyzing cell-mediated cytotoxicity

To demonstrate the flexibility of PAINTKiller, the NUS researchers combined it with a method known as cytokine secretion assay (CSA), which measures the proteins called cytokines that are released by immune cells during an immune response. During this experiment, the team simultaneously labeled the surface of killer cells with the receptors that can capture CFSE and cytokines which are important cell-signaling proteins.

Results from this experiment showed that PAINTKiller is a powerful tool for enabling comprehensive and flexible detection of cell-mediated cytotoxicity, protein secretion and surface receptors of individual killer cells in a high-throughput manner.

Given the complexity of various mechanisms that come into play during cell-mediated cytotoxicity, a multimodal, high-throughput single-cell system that can measure the cytotoxic capability, outline the characteristics of immune cells and proteins, as well as analyze gene expression will be useful for studying cell-mediated cytotoxicity accurately.

To this end, the NUS team pushed the boundaries of the PAINTKiller method and developed the single-cell PAINTKiller-seq assay, which is a single-cell sequencing workflow that integrates data from transcriptomic analysis, phenotypic analysis and cytotoxicity studies.

This workflow adds to the standard PAINTKiller method to investigate, at the molecular level, the characteristics of CFSE-labeled killer cells to understand whether they are genetically different from killer cells not labeled with CFSE. With the PAINTKiller-seq assay, the NUS

researchers conducted an in-depth analysis of the molecular factors that correlate with killer cell activity during cell-mediated cytotoxicity.

"The versatility of PAINTkiller holds significant promise for enhancing cell-based immunotherapy and advancing cell-manufacturing workflows. This innovative approach would provide a useful tool to comprehensively elevate the quality and functionality of the ultimate therapeutic product, marking a notable advancement in the field," said Dr. Wu.

The NUS team is working to expand the versatility of the PAINTkiller method by testing it on different killer and foreign cell combinations to reveal the biology behind the complexities of immune responses, and to develop it as an integral tool for research and clinical applications.

**More information:** Yen Hoon Luah et al, Identification, sorting and profiling of functional killer cells via the capture of fluorescent target-cell lysate, *Nature Biomedical Engineering* (2023). DOI: 10.1038/s41551-023-01089-z

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