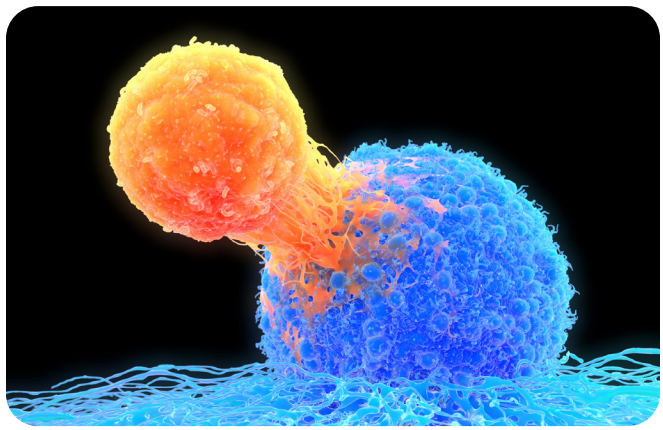
PUBLICATION REVIEW

Morphological profiling of human T and NK Lymphocytes by high-content imaging

Advances in high-content cellular imaging (HCI) and analysis have enabled researchers to obtain rich, quantitative data from cells and identify biologically relevant similarities and differences in their morphological profiles. Morphological profiling has been employed in cancer and toxicology research but has not been widely explored for the study of immune cells to date. However, because immune cell populations adopt distinctive morphologies and structures to sustain their various immuno-surveillance tasks, HCI is a useful technique for monitoring immune cell remodeling upon activation.

A critical event for the generation of antigen-specific immune responses is the formation of immunological synapses (IS) between immune cells and antigen-presenting cells. This process is particularly important for effector functions of cytotoxic lymphocytes such as CD8+ T cells and natural killer (NK) cells. IS assembly involves dynamic rearrangements of the actin cytoskeleton and recruitment of various adhesion molecules. Thus, imaging of the IS presents a unique window of opportunity to monitor the responsiveness and effector potential of various cytotoxic lymphocyte populations following activation. Such an approach could be useful for gaining a deeper understanding of lymphocyte biology, how this relates to immunodeficiencies, and for immunotherapeutic drug screening. It could also help guide the engineering of novel chimeric antigen receptors (CARs) since efficient IS formation is also important for CAR T cell effector function.



Summary

The present study provides a foundation for the development of morphological profiling as a scalable approach to monitor primary lymphocyte responsiveness and to understand lymphocyte microarchitecture. Morphological profiling could also provide novel insights into the immunological synapses (IS) of CAR T cells and guide the design of novel CARs for therapeutic applications.

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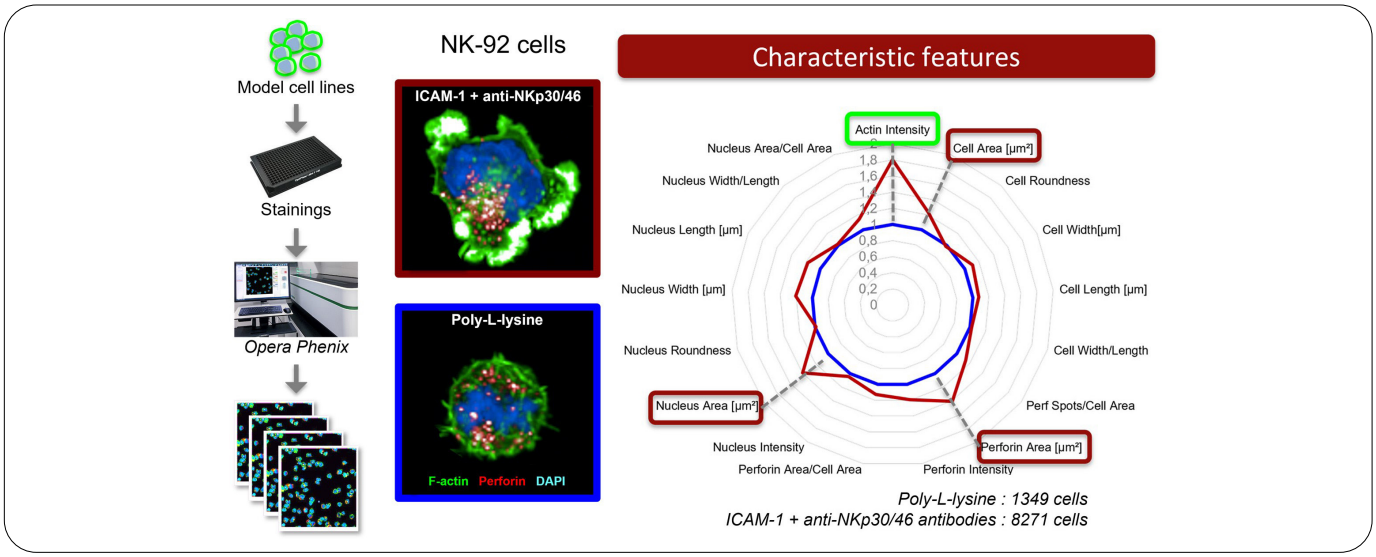

Figure 1: Overview of the HCI workflow for the immune synapse in lymphocytic cell lines. Image credit: Yolla German.

Morphological profiling of T and NK cell immunological synapses

In a recent study, researchers working with Loïc Dupré (Toulouse Institute for Infectious and Inflammatory Diseases, France and Medical University of Vienna, Austria) developed a HCI and computational image analysis workflow to evaluate the morphological profiles of cytotoxic T and NK lymphocyte ISs.[1] The aim was to detect morphological traits of lymphocyte populations upon activation and understand lymphocyte microarchitecture in the context of diseases such as inborn errors of immunity. As proof of concept, the researchers first applied their approach to NK-92 and Jurkat cells, two human cell lines commonly used as models for NK cells and T cells, respectively. They then explored the applicability of their approach to primary human cytotoxic cell subsets. Figure 1 provides an overview of the HCI pipeline.

Once the researchers had validated their approach, they set out to determine how actin cytoskeleton remodeling contributed to IS assembly. This was achieved by comparing morphological alterations of NK cell ISs following treatment with a series of actin-targeting drugs. NK-92 cells were stimulated with ICAM-1 and anti-NKp30/NKp46 antibodies and then treated with three concentrations of the drugs latrunculin B, jasplakinolide, blebbistatin, Y-27632, CK-869, wiskostatin, and SMIFH2. As shown in Figure 2, morphological profile analysis revealed that the distribution of lytic granules (detected via a perforin staining) at the NK IS is dependent on the integrity of various facets of actin organization (stained with phalloidin), supporting the notion that multiple actin-dependent steps control lytic granule docking and exocytosis.

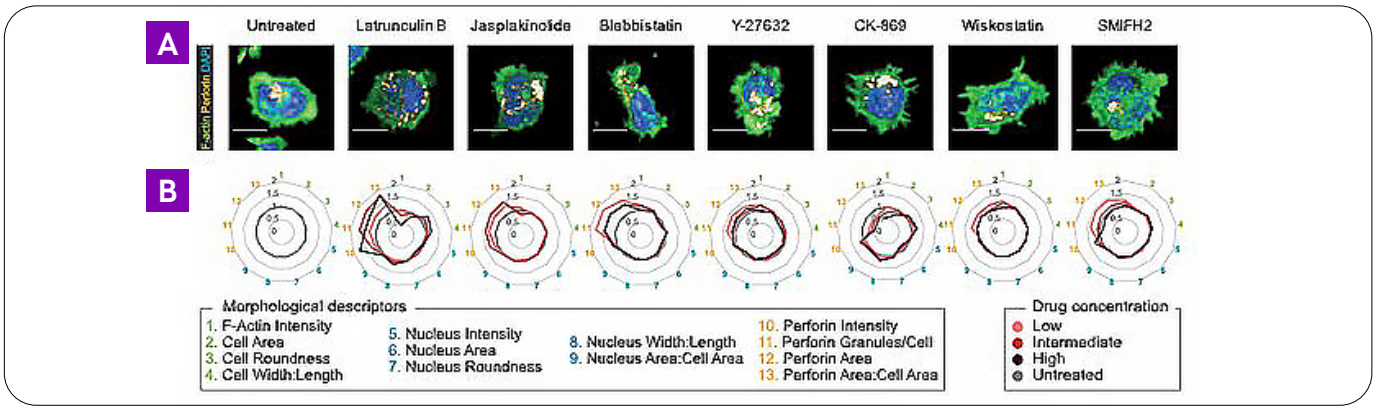

Figure 2: Comparative analysis of the effect of actin-targeting drugs on lytic granule distribution and exocytosis in NK-92 cells. Representative images (A) and graphs (B) representing the fold change of IS descriptors for NK-92 cells seeded on ICAM-1, anti-NKp30, and anti- NKp46, stained for F-actin (green), perforin granules (yellow), and nuclei (blue) and treated with three concentrations of latrunculin B, jasplakinolide, blebbistatin, Y-27632, CK-869, wiskostatin, and SMIFH2 with respect to the untreated control. Image credit: German, Yolla et al. 2021

The team then expanded their set of morphological descriptors to include a total of 383 measurements. This further enriched their results and enabled the identification of relevant spatially localized events and characterization of perturbed cell states.

Morphological profiling of primary human NK cells and CD8+ T cells

Finally, when the researchers applied their pipeline to lymphocytes isolated from the peripheral blood of three healthy donors, they found that their HCI approach could discriminate between individual donors on the basis of immune cell morphological traits. They also demonstrated the clinical applicability of their approach by identifying aberrations in IS assembly and lytic granule polarization in individuals with severe congenital inborn errors of immunity. HCI-based morphological profiling was particularly adapted to discriminate alterations in closely related disease entities, highlighting its potential to provide unique patient signatures to assist diagnosis and guide searches for disease mechanisms.

Conclusion

The present study provides a foundation for the development of morphological profiling as a scalable approach to monitor primary lymphocyte responsiveness and to understand lymphocyte microarchitecture. Morphological profiling could also provide novel insights into the IS of CAR T cells and guide the design of novel CARs for therapeutic applications.

The researchers showcase how various human lymphocyte populations, including model cell lines, cells freshly isolated from the blood, and expanded primary cells can be activated with plate coatings, stained, and then imaged with an automated confocal microscope at high resolution in a 384-well format. They also demonstrate how several samples, activation conditions, and perturbations can be successfully analyzed in parallel. Furthermore, because of the distinct morphological profiles observed for each drug tested and the dose-dependent effects in cell lines and primary cells, the researchers suggest that this approach could be applied to immunotherapeutic drug screening workflows in the future.

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"The Opera Phenix system has allowed us to standardize and greatly increase the throughput of immunofluorescence on immune cells, such as T cells and NK cells. Automated analysis of thousands of cells dispensed over various stimulatory molecules in 384-well plates and stained with various dyes and antibodies yields rich datasets that open new avenues for diagnostic, disease mechanism exploration, therapeutic T cell optimization, and drug screens."

Reference

1. German, Yolla et al. 2021. "Morphological Profiling of Human T and NK Lymphocytes by High-Content Cell Imaging." *Cell reports* 36(1):109318.