PRELIMINARY STUDY

Samplix

Combined single-cell functional analysis and molecular profiling of Tumor Infiltrating Lymphocytes

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Summary

- Tumor Infiltrating Lymphocytes (TILs) from solid tumors can be characterized and isolated based on their cytotoxic activity.
- Thousands of functional, paired T-cell Receptor (TCR) α- and β-chain sequences can be obtained.
- High-throughput droplet-based co-encapsulation of TILs and tumor cells enables functional characterization and sequencing, potentially revealing neoantigens and escape mutations.

Introduction

Adoptive T cell therapy is a promising approach in cancer immunotherapy, yet challenges remain in predicting T cell potency and improving treatment efficacy. A key issue is the heterogeneity of T cell cytotoxicity, which contributes to inefficiencies. Current methods to address this limitation face significant challenges, as they lack the ability to provide functional analysis at single-cell resolution[1].

Single-cell technologies overcome these limitations by enabling the dissection of T cell heterogeneity and identifying therapeutically relevant clones[2]. Droplet microfluidics allows for precise single-cell encapsulation, facilitating functional and molecular profiling of immune cells.

Kiel-Rasmussen *et al.* demonstrated the power of double-emulsion droplet technology for analyzing key cell types used in adoptive cell therapy, including natural killer cells, tumor-infiltrating lymphocytes (TILs), and CAR T cells[3]. Here, we extend this approach to demonstrate combined single-cell functional analysis and single cell transcriptome profiling of active and non-active TILs co-encapsulated with matched patient tumor cells.

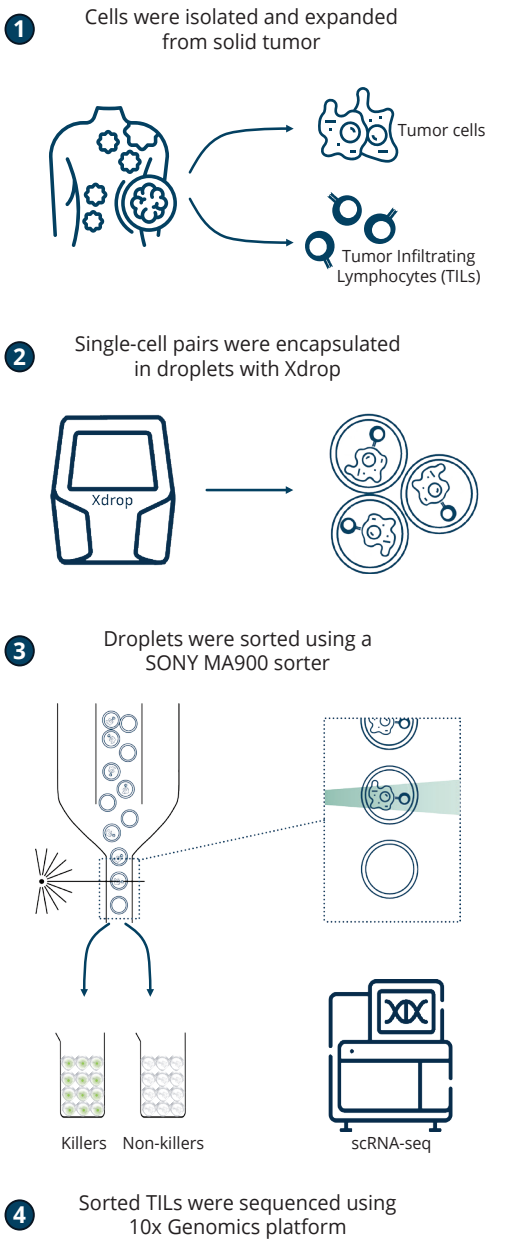

Figure 1. The workflow shows the isolation of tumor cells and tumor infiltrating lymphocytes (TILs) from a solid tumor. These cells were co-encapsulated using Samplix's Xdrop platform to assess cytotoxic activity at the single-cell level. Sorted populations were sequenced using the 10x Genomics platform.

Tumor cells and TILs co-encapsulated in droplets

Matched expanded TILs and tumor cells from the same patient were obtained from the National Center for Cancer Immune Therapy (CCIT-DK) at Herlev Hospital. Dead cells were removed by magnetic cell isolation (MACS, Miltenyi) and TILs were stained with CellTrace Violet (Thermo Fisher Scientific) (1:5000), while tumor cells were stained with CellTrace Far Red (Thermo Fisher Scientific) (1:5000). Cells were mixed at a 1:2.25 TIL:tumor ratio in R10 media with Incucyte Caspase-3/7 Green Dye (Satorius) (1:1000) and immediately encapsulated in Xdrop D50 droplets. After three hours of incubation, droplets were sorted on a SONY MA900 cell sorter.

Functional and molecular profiling of T-cells

Droplets triple-positive for TILs, tumor cells, and caspase-3/7 were classified as "Killers," while those positive for TILs and tumor cells but not caspase-3/7 were "Non-killers." Cells were recovered from sorted droplets using Droplet Break Solution (Samplix) and processed using the 10X Chromium platform for both gene expression and T-cell Receptor (TCR) sequencing, followed by sequencing on Illumina NovaSeq 6000.

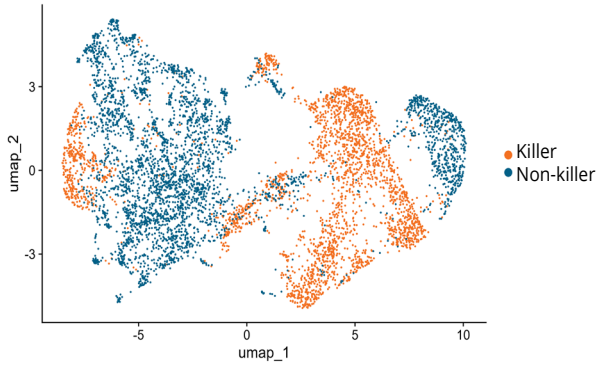

Figure 2. UMAP visualization of single-cell transcriptomic data overlayed with Xdrop generated single-cell functional assessment of cytotoxicity. The figure includes data from TILs only, excluding cancer cells. Each dot represents a single cell.

This preliminary study demonstrates how Xdrop single-cell functional analysis can be combined with single-cell transcriptomics and TCR sequencing. In this particular patient sample, TILs were separated based on their ability to kill or not kill a cancer cell. In total, gene expression profiles were obtained from 5,548 "Killer" and 11,391 "Non-killer" cells (mix of TILs and tumor cells). After filtering for CD3+ expression, >2,700 T cells remained in each group. Single-cell transcriptomics identified two main groups of cells (Figure 2).

Interestingly, overlaying this data with Xdrop generated single-cell functional data on TIL cytotoxicity, shows that the two groups mainly represent "Killers" and "Non-killers". Subpopulations of each group were also observed (Figure 2), possibly indicating killers that did not yet kill and non-killers co-encapsulated with a dead cancer cell.

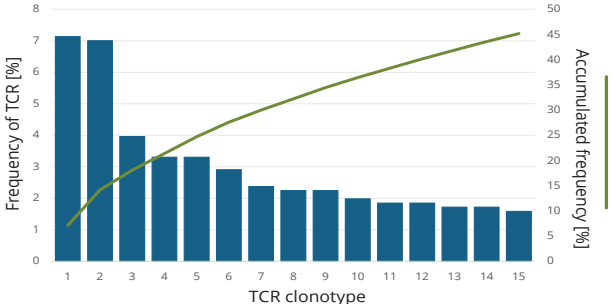

Figure 3. Shown is the most frequent T-celll Receptor (TCR) clonotypes identified by functional single-cell screening followed by single-cell sequencing.

TCR sequencing identified >240 different TCRs in the "Killer" TIL population. The 15 most frequent clonotypes overall account for >45% of the identified TCRs (Figure 3). Future studies will reveal the similarities and differences in TCR populations between killers and non-killers.

Conclusion

Co-encapsulation of immune and cancer cells enables high-throughput functional analysis and droplet sorting based on parameters such as cytotoxicity, caspase activation, granzyme B or cytokine release. This is followed by molecular profiling, including RNA sequencing. Further investigation aims to uncover its full potential.

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References

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