WEBINAR SUMMARY

Transforming Brain Metastasis Research: Levitation Technology Enables High-Quality Single Cell Analysis

Webinar Presented by Jan Remsik

Jan Remsik, PharmD, PhD, is a postdoctoral fellow in the Adrienne Boire lab at Memorial Sloan Kettering Cancer Center in New York. Through developing syngeneic animal models of brain metastasis and derived single-cell datasets, Jan studies the interactions between cancer and immune system to develop novel immunotherapeutics against metastasis in this space.

Brain Cancer Research: Untangling the Complexity of Leptomeningeal Metastasis

Leptomeningeal metastasis (LM) is a rare, late complication of cancer characterized by spread of cancer cells into the cerebrospinal fluid (CSF). CSF liquid biopsy samples from LM subjects contain numerous cell subtypes. Obtaining and characterizing the viable cells from all subtypes at physiological representation is an arduous task due to the challenging nature of these samples, including:

- Low cell counts
- Extreme fragility of the cells
- Debris

This is further compounded by standard methods for sample quality improvement that either result in no live cell yield from low cell count samples or modify levels of RNA and proteins within cells, confounding scientific conclusions.

The LeviCell® system provides a rapid and gentle viable cell enrichment method that was successfully employed to generate scRNA-seq and CITE-seq data from CSF samples, obtaining unprecedented results in regards to quality and sensitive cell subtype representation.

No Stress: High-Quality Cell Data From Challenging Samples

A total of 24 mice were used to prepare four sample pools for sequencing using cell hashing. The pools were enriched for viability on the LeviCell 1.0 system and

KEY HIGHLIGHTS

The LeviCell 1.0 system optimizes the processing of challenging samples

- Viability improvement of fragile samples from 72% to 97%
- 85% of all single cells pass high-quality data filters
- Accurate cell subtype representation of even the most fragile ones like dendritic cells and neutrophils

85% of all sequenced droplets passed stringent filters applied. The high retention of single-cells in the final dataset resulted in accurate cell subtype representation across all models tested (Figure 1).

Preserving Cell States: A Critical Factor in Data Reliability

A bonus of the gentle processing of the LeviCell system is the ability to maintain native cell states. In the downstream analysis, an average of only 3% of the single-cell reads mapped to the mitochondrial genome, indicating that the applied workflow effectively minimized cell-stress responses. Furthermore, there was a lack of combined expression of the stress markers JUN and FOS in the dataset, which normally indicates cell stress (Figure 2). Specific expression of either of these genes was limited to the cell subtypes containing signaling pathways in which the respective genes participate. These findings suggest that the sample processing pipeline, including the LeviCell 1.0 system, does not induce cell stress, thereby ensuring the reliability of the data generated.

The impact of preserving cell native states during viability improvement is clear: fragile cell subtypes, such as dendritic cells and neuthrophils, are represented in the data even after rigorous quality filters are applied.

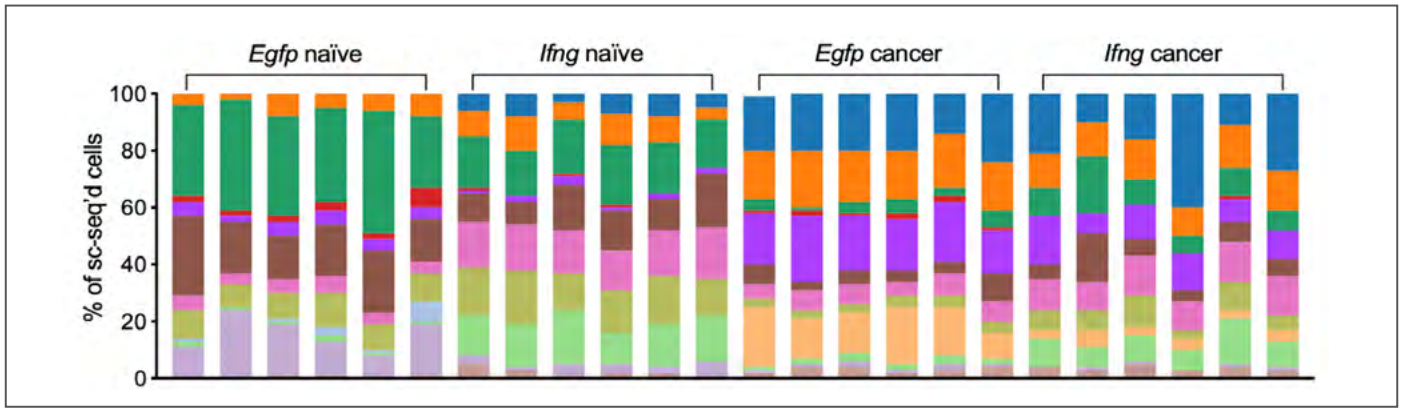

Figure 1. Cell composition of mouse CSF samples is preserved by the LeviCell 1.0 system. *Mouse CSF from four different conditions was collected and processed in six replicates, including naive mice and immunocompetent model of mouse lung LM. Cell subtype representation (different colors) was preserved across replicates of the same mouse model and reflected the composition of previous data from these CSF samples, profiled with routine cytometry.*

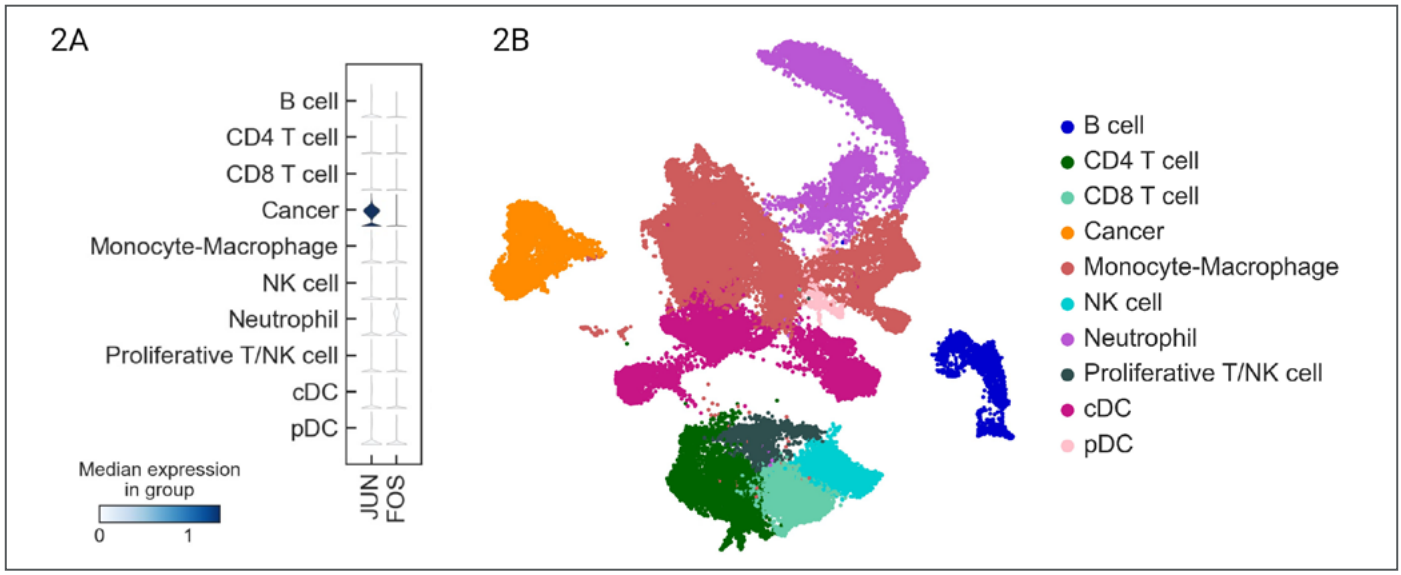

Figure 2. The LeviCell 1.0 system does not increase cellular stress during sample preparation. *A. Violin plots show expression levels of JUN and FOS, two markers of cell stress. No cell subtype was detected as expressing both genes, which would be expected in stressed cells. B. UMAP of integrated scRNA-seq and CITE-seq data collected from mouse LM models showing presence of sensitive cell subtypes such as dendritic cells and neutrophils. (Remsik et al. (2023). Leptomeningeal anti-tumor immunity follows unique signaling principles. bioRxiv.)*

Conclusions

The ability of the LeviCell system to rapidly process challenging CSF samples without inducing cell stress led to high-quality data and accurate preservation of fragile cell subtypes such as dendritic cells and neutrophils. The unprecedented insights achieved by this process improve our understanding of CSF sample composition.

In future studies, Jan and collaborators plan to include ATAC-seq employing the LeviCell EOS system for extended multi-omics analyses of the LM disease. Compared to the LeviCell 1.0 system, the LeviCell EOS system will provide a high-throughput sample quality improvement for 4x faster data generation.