Single-cell RNA Sequencing Reveals Intrathecal Treatment Sensitivity and Regulatory Mechanisms in Breast Cancer Leptomeningeal Metastasis

Jizhuo Gao[1]; Haoran Li[1]; Shanshan Zhao[1]; Man Li[1]; Lingzhi Xu[1]
[1]Department of Oncology, The Second Affiliated Hospital of Dalian Medical University, Dalian, China

Abstract

Breast cancer leptomeningeal metastasis (BCLM) is a devastating complication characterized by rapid progression and poor prognosis. Intrathecal therapy, which delivers anticancer agents directly into the cerebrospinal fluid (CSF), serves as a critical localized treatment. However, patients exhibit marked heterogeneity in therapeutic response, and the underlying cellular mechanisms remain poorly understood.

To elucidate these mechanisms, we performed single-cell RNA sequencing (scRNA-seq) on CSF cells from BCLM patients, aiming to comprehensively characterize the tumor microenvironment and identify cellular subpopulations associated with treatment sensitivity. Our analysis revealed a reconfigured immune landscape and identified a distinct monocyte/macrophage subcluster (C3_S100A9$^+$) that was significantly enriched in treatment-sensitive patients. This subpopulation exhibited a unique phenotype characterized by inflammatory activation, elevated ribosomal protein expression, and extensive interactions with highly malignant tumor cells.

These findings provide new insights into the cellular basis of differential treatment response in BCLM. The identified C3_S100A9$^+$ macrophage subset represents a promising predictive biomarker and a potential therapeutic target, paving the way for more precise management strategies for this challenging condition.

Introduction

Breast cancer leptomeningeal metastasis (BCLM) affects a subset of advanced breast cancer patients and represents one of the most challenging clinical complications due to rapid disease progression, neurological decline, and limited treatment options[1,2]. Intrathecal therapy[3], which delivers chemotherapeutic agents directly into the CSF, can achieve local tumor control and improve patient outcomes. Despite this, response rates are highly variable, and current clinical parameters are insufficient to predict which patients will benefit. Understanding the cellular and molecular features of the CSF tumor microenvironment (TME) is crucial for deciphering mechanisms of therapeutic resistance and sensitivity. Recent advances in single-cell RNA sequencing (scRNA-seq) enable high-resolution characterization of complex cellular ecosystems, allowing identification of rare subpopulations and their functional states[4,5]. In this study, we aimed to comprehensively map the CSF TME in BCLM patients, delineate epithelial and immune cell heterogeneity, and identify key cellular and molecular determinants of intrathecal therapy response.

Methods and Materials

Samples: CSF was collected from eight BCLM patients (categorized as pre-treatment, post-treatment sensitive, and post-treatment resistant). Four normal CSF samples from GEO served as controls (Table 1).

Sequencing: Single-cell RNA sequencing was performed using the 10X Genomics platform, yielding 80,274 high-quality cells after quality control (Figure 1).

Analysis: Dimensionality reduction, clustering, CNV analysis, pseudotime trajectory inference, and cell-cell interaction analyses were applied to define cellular subtypes and functional states (Figure 1).

Sample Name	Intrathecal Therapy	Sensitivity Assessment	Source
PreT1	NO	/	Dept. of Breast Oncology, Second Affiliated Hospital of DMU
PreT2			
PreT3			
PreT4			
Te1	Yes	Effective	
Te2			
Tne1		Ineffective	
Tne2			
N1	Normal CSF as Negative Control		GEO Database
N2			
N3			
N4			

Table 1. Summary of Sample Information in This Study

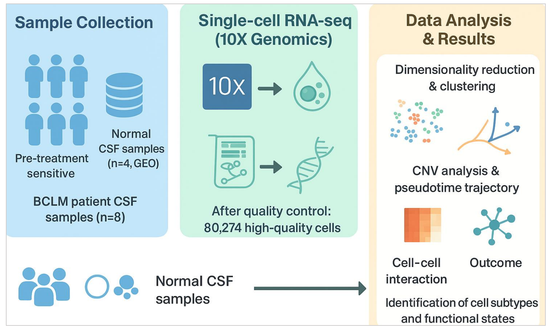


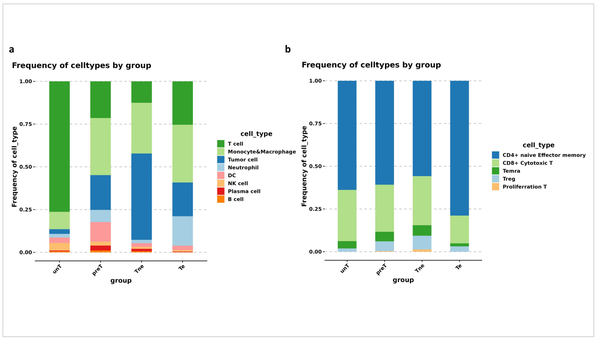
Figure 1. Workflow of single-cell RNA sequencing and analysis of cerebrospinal fluid (CSF) from BCLM patients.

Results

Immune landscape: Seven major immune populations were identified (T cells, monocytes/macrophages, neutrophils, dendritic cells, NK cells, plasma cells, and B cells) with treatment-dependent distribution changes. BCLM CSF exhibited reduced cytotoxic T cells and NK cells, increased Tregs, and M2-skewed monocytes/macrophages. Ribosomal protein gene expression was downregulated across multiple immune subsets, indicating an immunosuppressive TME (Figure 2a~b).

Epithelial/tumor cells: Epithelial cells were classified into seven heterogeneous subpopulations. CNV analysis distinguished high- versus low-malignancy subtypes, with high-malignancy cells showing elevated proliferative and migratory potential, possibly contributing to therapy resistance. Some epithelial subsets were also found in normal CSF, suggesting a non-malignant origin (Figure 2c).

Monocyte/macrophage subpopulations: Twelve transcriptionally distinct subclusters were identified. Notably, the C3_S100A9$^+$ subcluster was enriched in treatment-sensitive patients, showing high expression of inflammatory markers, ribosomal proteins, and regulatory lncRNAs. Pseudotime analysis positioned this subcluster at the terminal differentiation stage along the treatment-sensitive trajectory. Cell-cell interaction analysis revealed strong ligand-receptor crosstalk with highly malignant tumor cells, suggesting roles in adhesion, metabolism, and immune modulation (Figure 2d~f).



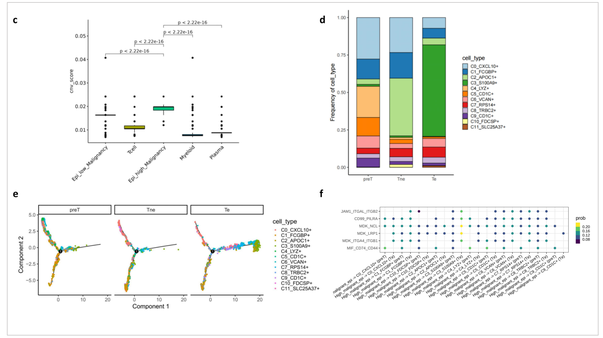


Figure 2. (a)cell subtype proportions of cerebrospinal fluid samples from 12 patients; **(b)** subtype proportions of T cells from 12 cerebrospinal fluid samples; **(c)** Boxplots of copy number variation (CNV) analysis in epithelial cells from the 12 cerebrospinal fluid samples; **(d)** cell subtype proportions of macrophages from BCLM patient samples; **(e)** Pseudotime trajectory analysis of macrophages from BCLM patient samples; **(f)** Ligand–receptor interaction analysis between macrophages and tumor cells.

Conclusions

- **High-resolution profiling:**

Single-cell transcriptomic mapping of CSF in BCLM reveals distinct cellular subpopulations associated with intrathecal therapy response.

- **Key immune signature:**

The C3_S100A9$^+$ monocyte/macrophage subcluster emerges as a potential predictive biomarker and therapeutic target, capable of modulating immune activation and tumor interactions.

- **Toward precision therapy:**

Integrative single-cell and functional analyses provide a foundation for precision intrathecal therapy and patient stratification in BCLM.

- **Next steps:**

Ongoing work focuses on transcription factor networks and mechanistic validation to uncover immune regulatory pathways and guide the development of novel immunomodulatory strategies.

Contact

LingzhiXu
The Second Affiliated Hospital of Dalian Medical University, Dalian, China
Email: xvlignzhi@sina.com

References

[1] SCOTT B J, OBERHEIM-BUSH N A, KESARI S. Leptomeningeal metastasis in breast cancer - a systematic review [J]. Oncotarget, 2016, 7(4): 3740-7.
[2] FAYERS P, BOTTOMLEY A, GROUP E Q O L, et al. Quality of life research within the EORTC-the EORTC QLQ-C30. European Organisation for Research and Treatment of Cancer [J]. Eur J Cancer, 2002, 38 Suppl 4: S125-33.
[3] GLANTZ M J, VAN HORN A, FISHER R, et al. Route of intracerebrospinal fluid chemotherapy administration and efficacy of therapy in neoplastic meningitis [J]. Cancer, 2010, 116(8): 1947-52.
[4] CHIOSSONE L, DUMAS P Y, VIENNE M, et al. Natural killer cells and other innate lymphoid cells in cancer [J]. Nat Rev Immunol, 2018, 18(11): 671-88.
[5] TIROSH I, IZAR B, PRAKADAN S M, et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq [J]. Science, 2016, 352(6282): 189-96.

This presentation is the intellectual property of the author/presenter. Contact them at xvlignzhi@sina.com for permission to reprint and/or distribute.

© Poster Template by Genigraphics® 1.800.790.4001 www.genigraphics.com