

Case Report

Case report: single-cell characterization of cerebrospinal fluid in a lung squamous cell carcinoma patient with brain metastasis

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Abstract: Brain metastasis (BrM) originating from lung squamous cell carcinoma (LUSC) confers a dismal prognosis due to limited therapeutic options and immunotherapy resistance. Here, we report a 70-year-old man with advanced LUSC and BrM who experienced intracranial progression during camrelizumab-based chemoimmunotherapy and subsequently received radiotherapy and modified chemotherapy, with an overall survival of five months. Single-cell RNA sequencing (scRNA-seq) of a cerebrospinal fluid sample, integrated with public primary and metastatic datasets, identified a CD8⁺ T-cell cluster enriched for a stress-response program. Exploratory analyses of public transcriptomic cohorts suggested an association between a higher stress response T-cell (Tstr) signature and unfavorable outcomes.

Keywords: CD8⁺ stress response T cell, single-cell RNA sequencing, lung squamous cell carcinoma, brain metastasis, immunotherapy, case report

Introduction

Brain metastasis (BrM) is a frequent and lethal complication of non-small-cell lung cancer (NSCLC), substantially reducing patients' overall survival (OS). BrM arising from lung squamous cell carcinoma (LUSC) is less common than that arising from lung adenocarcinoma (LUAD) but is associated with a poor prognosis and remains insufficiently characterized [1, 2]. Treatment is complicated by the blood-brain barrier (BBB) and by immune remodeling within metastatic lesions. Compared with primary tumors, BrM lesions often display an immunosuppressive and heterogeneous microenvironment that may facilitate immune escape and limit responses to immune-checkpoint inhibitors (ICIs) [3, 4].

Single-cell RNA sequencing (scRNA-seq) enables the identification of cellular states and transcriptional programs that are obscured by bulk profiling. Thus, in this study, we analyzed

cerebrospinal fluid (CSF) from a patient with LUSC and concurrent BrM who subsequently experienced intracranial progression despite multimodal treatment. By integrating these data with published scRNA-seq datasets of primary LUSC and BrM lesions, we sought to define the cellular and immune landscape of LUSC BrM and investigate mechanisms associated with intracranial progression and treatment resistance.

Case description

Initial diagnosis and treatment response

A 70-year-old man was admitted on March 28, 2024, with recurrent hemoptysis accompanied by dark-red blood clots. The clinical course is summarized in **Figure 1A**. Contrast-enhanced chest computed tomography (CT) revealed a left upper-lobe mass, multiple enlarged mediastinal lymph nodes and a left pleural effusion (**Figure 1B**). CT-guided percutaneous needle

Single-cell characterization of cerebrospinal fluid

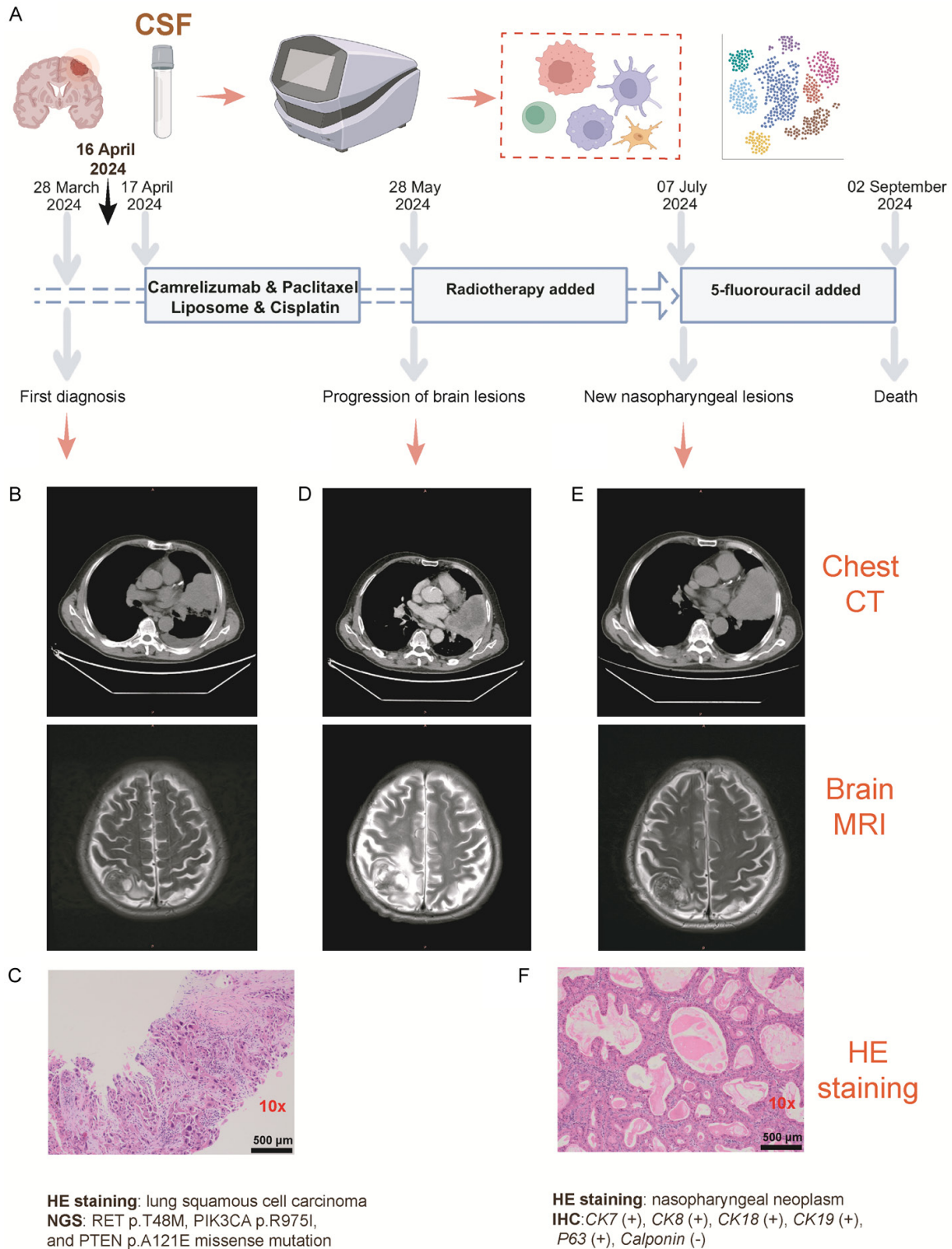


Figure 1. Clinical course, imaging, and pathological findings of a 70-year-old man with lung squamous cell carcinoma (LUSC) and brain metastasis (BrM). **A.** Schematic overview of the clinical course, including diagnosis, cerebrospinal fluid (CSF) sampling, treatment modifications, disease progression, and outcome. **B.** Baseline chest computed tomography (CT) and brain magnetic resonance imaging (MRI) showing a left upper-lobe lung lesion and a parietal lobe lesion. **C.** Hematoxylin and eosin (H&E) staining of the lung biopsy specimen showing poorly differentiated LUSC with necrosis. Next-generation sequencing (NGS) identified RET p.T48M, PIK3CA p.R975I, and PTEN p.A121E variants. 10× objective; scale bar, 500 μm. **D.** Chest CT and brain MRI scans showing progression,

especially brain lesions, after 1.5 months of the application of camrelizumab with paclitaxel liposome and cisplatin. E. Follow-up imaging showing relative stabilization of the pulmonary and intracranial lesions. F. H&E staining of the nasopharyngeal neoplasm and immunohistochemistry (IHC) staining results for CK7, CK8, CK18, CK19, P63 and calponin. 10× objective; scale bar, 500 µm.

biopsy confirmed moderately to poorly differentiated LUSC with necrosis, as shown by hematoxylin and eosin (H&E) staining (**Figure 1C**).

Brain magnetic resonance imaging (MRI) revealed a parietal lesion consistent with BrM, together with additional abnormalities in the cerebral white matter (**Figure 1B**), supporting the diagnosis of LUSC with BrM. The disease was classified as stage IVA (T4NxM1b) and was considered unsuitable for curative surgery.

Next-generation sequencing identified RET p.T48M, PIK3CA p.R975I, and PTEN p.A121E variants. From April 17 to May 28, 2024, the patient received camrelizumab combined with liposomal paclitaxel and cisplatin. Follow-up imaging showed enlargement of the intracranial lesion, consistent with progressive disease (PD) (**Figure 1D**).

Subsequent treatment and disease progression

Owing to progression of the parietal metastasis, palliative radiotherapy was administered from May 28 to July 19, 2024. During treatment, new nasopharyngeal lesions developed (**Figure 1F**). Immunohistochemistry (IHC) showed positive staining for CK7, CK8, CK18, CK19 and P63, but negative staining for calponin. Because the lesions invaded the skull base and caused nasal obstruction, 5-fluorouracil was added on July 7, 2024. After two additional cycles of chemoimmunotherapy, follow-up imaging showed relative stabilization of the pulmonary and intracranial lesions (**Figure 1E**).

Despite multimodal treatment, the patient's condition subsequently deteriorated without substantial regression. Further progression of the parietal metastasis precluded additional treatment, and the patient died on September 2, 2024, approximately five months after diagnosis. No severe acute treatment-related adverse events occurred. Fatigue and nausea were manageable with supportive care.

Single-cell transcriptomic analysis

To characterize CD8⁺ T-cell states in primary and metastatic LUSC, we collected scRNA-seq

data from a BrM lesion (GSM5416355), four primary LUSC samples (E-MTAB-13526), and one CSF sample acquired from the patient. Detailed methods for single-cell and bioinformatic analyses are provided in the [Supplementary Methods](#).

Single-cell atlas and characterization of CD8⁺ T-cell states

After integration and quality control, 24,719 cells with 25,925 detected features were retained and grouped into 14 distinct clusters (**Figure 2A**). Based on CD2 and CD8A expression, 9 clusters comprising 11,815 cells were annotated as CD8⁺ T cells (**Figure 2B, 2C**). We initially evaluated module scores for 12 predefined T-cell states: natural killer T cell (NKT), proliferation T cell, quiescence T cell, senescence T cell, cytotoxic T cell (Tc), central memory T cell (Tcm), effector memory T cell (Tem), terminally differentiated effector memory T cell (Temra), exhausted T cell (Tex), naïve T cell (Tn), tissue-resident memory T cell (Trm) and stress response T cell (Tstr). We also examined the expression of representative marker and functional genes across clusters (**Figure 2D, 2E**). Functional scoring and marker-gene expression identified 8 major CD8⁺ T-cell categories: NKT, proliferation T cell, quiescence T cell, senescence T cell, Tc, Tem, Tn and Tstr (**Figure 2F**).

To further assess the Tstr annotation, we calculated module scores using previously reported stress-response-associated gene signatures [5, 6]. Module scoring showed higher Tstr signature scores in the annotated CD8⁺ Tstr population (**Figure 2G**). Tissue-enrichment analysis based on observed to expected (Ro/e) ratios showed preferential enrichment of Tem, Tstr, and Tn in BrM and CSF (**Figure 2H**). Subsequently, we conducted functional enrichment analysis and found distinct biological programs across the CD8⁺ T-cell states (**Figure 2I**).

Association of CD8⁺ Tstr-related signatures with clinical outcomes

To explore the clinical relevance of CD8⁺ Tstr-related signatures, we compared T-cell-state scores between responders and non-respond-

Single-cell characterization of cerebrospinal fluid

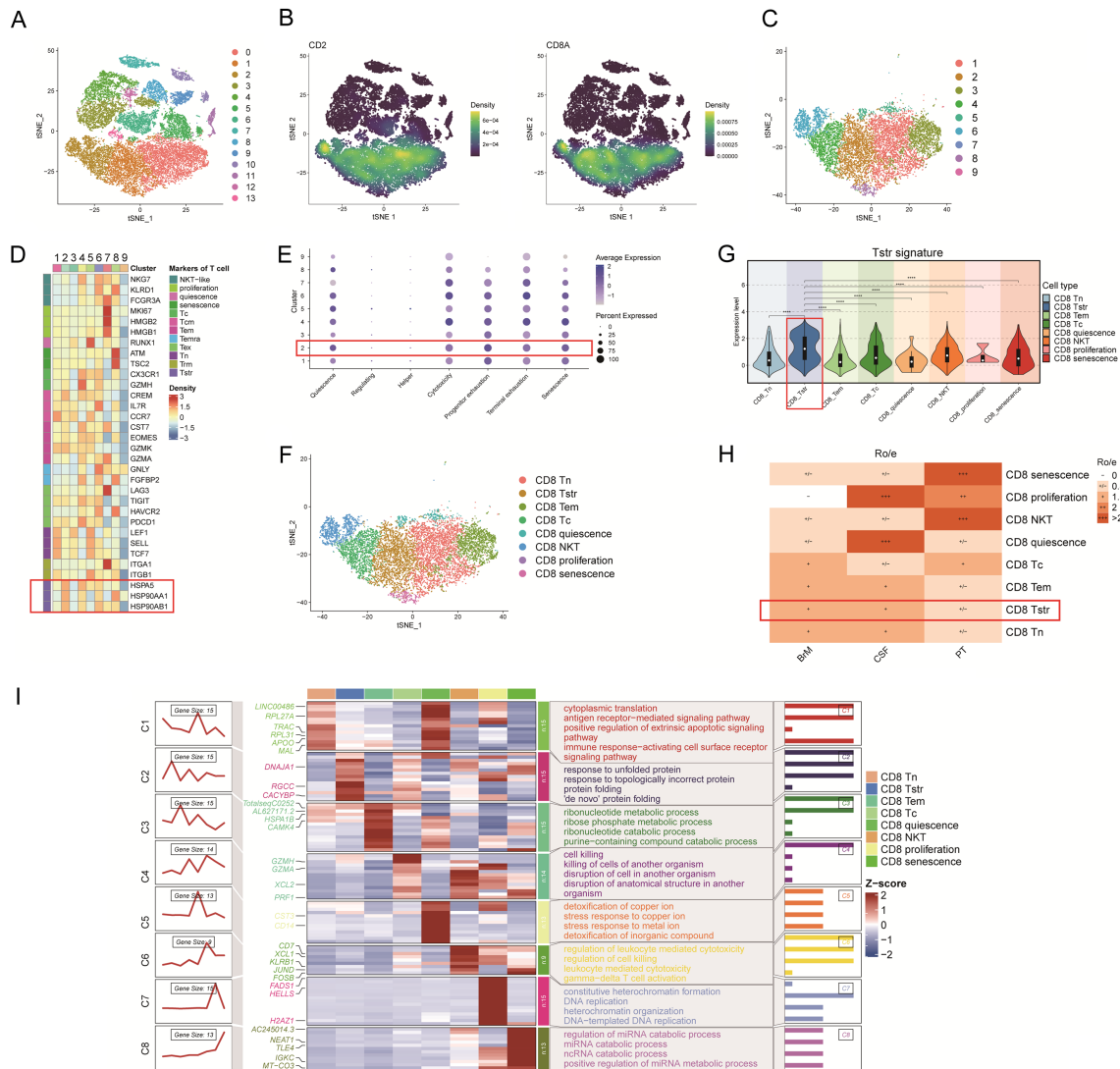


Figure 2. Single-cell characterization of CD8⁺ T-cell states across primary and metastatic LUSC samples. A. A t-distributed stochastic neighbor embedding (t-SNE) plot depicting the distribution of 14 cell clusters within the integrated single-cell milieu. B. t-SNE visualizations of scRNA-seq data, highlighting the distribution of CD2- and CD8A-positive cells. C. A t-SNE visualization of clusters belonging to CD8⁺ T-cell states. D. A heatmap depicting the expression levels of representative marker genes used for CD8⁺ T-cell state annotation. E. A dot plot showing functional module scores across CD8⁺ T-cell clusters. F. A t-SNE plot showing the distribution of 8 annotated CD8⁺ T-cell states: naïve (Tn), stress-response (Tstr), effector memory (Tem), cytotoxic (Tc), natural killer T (NKT), quiescence, proliferation, and senescence states. G. Module-score analysis showing enrichment of the stress- and exhaustion-related gene signature in the annotated CD8⁺ Tstr population. H. A heatmap showing tissue enrichment of different CD8⁺ T-cell states based on observed to expected (Ro/e) ratios across brain metastasis (BrM), cerebrospinal fluid (CSF), and primary-tumor compartments. I. A heatmap presenting a comprehensive analysis of CD8⁺ T-cell populations and their associated biological functions.

ers in the IMvigor210 immunotherapy cohort (Figure S1A). Tstr-related scores were higher in patients with stable or progressive disease (SD/PD) than in those with complete or partial response (CR/PR) ($P < 0.05$). Additionally, Kaplan-Meier (KM) analyses across the IMvigor210, TCGA-LUSC, GSE74777, combined GSE-

37745/GSE73403, and GSE30219 cohorts demonstrated that higher Tstr-related scores were associated with shorter survival (Figure S1B-F) (all $P < 0.05$). In the TCGA-LUSC cohort, the Tstr-high group exhibited higher immune and stromal scores and lower tumor mutation burden (TMB) (all $P < 0.05$) (Figure S1G-I).

Tstr scores were generally higher in BrM than in matched primary lung tumors in both LUAD and LUSC subtypes in the GSE161116 cohort (Figure S1J). In the Yang et al. NSCLC BrM cohort, multivariable Cox analysis showed that Tstr-high status (HR = 1.65, 95% CI: 1.02-2.66; *p*-value = 0.041) and age $\geq$ 60 years (HR = 1.73, 95% CI: 1.06-2.82; *p*-value = 0.029) were associated with shorter OS (Figure S1K).

CellChat analysis of predicted intercellular communication

CellChat analysis was used to characterize the strength and number of interaction signals between different cell clusters (Figure S2A). A scatter plot was generated to quantify the incoming and outgoing signals for each cell cluster (Figure S2B). To identify signal differences between distinct tissue types, we conducted a detailed investigation of signaling patterns and ligand-receptor (L-R) pairs. Comparative analysis predicted prominent interactions between CD8⁺ Tstr and Tc cells, including HLA-CD8-associated L-R interactions (Figure S2C). Comparison across tissue types showed increased predicted MHC-I- and MIF-associated signaling in Tstr from BrM and CSF, whereas CLEC-associated signaling was more prominent in primary LUSC samples (Figure S2C-F). These results indicate tissue-dependent variation in the predicted intercellular communication of CD8⁺ Tstr.

Molecular characterization of pretreatment CSF

To provide clinical and molecular identification for the CSF, we independently re-analyzed its scRNA-seq data. The final atlas comprised 10,080 cells, predominantly myeloid, CD4⁺ T, and CD8⁺ T cells, with smaller microglia, B-cell, and fibroblast populations (Figure S3A-C).

B cells expressed multiple immunoglobulin-associated transcripts, supporting a detectable humoral immune-related transcriptional program (Figure S3D), whereas tumor marker-related transcripts were generally sparse (Figure S3E). Cytokine-related profiling revealed prominent inflammatory transcriptional activity in myeloid cells, while IFNG and CCL5 were preferentially expressed in T-cell populations, particularly CD8⁺ T cells, consistent with their effector-associated transcriptional pheno-

type (Figure S3F). These findings suggest that the pretreatment CSF was characterized by a myeloid-dominant inflammatory transcriptional milieu accompanied by T-cell-associated IFN- γ /CCL5 signals.

Discussion

BrM from LUSC remains relatively uncommon, and effective therapeutic options are limited. In this study, we report a clinical case of a patient with LUSC and BrM. Given the difficulty of directly obtaining intracranial metastatic tissue, we collected CSF and performed scRNA-seq to explore the heterogeneity of CD8⁺ T-cell states.

Previous studies provide relevant biological and clinical context for our findings, as summarized in Table S1. First, CSF-based studies have demonstrated the feasibility of using CSF to characterize selected features of the intracranial TME. In patients with BrM, paired analyses of CSF and intracranial lesions revealed concordant patterns of CD8⁺ T-cell infiltration and shared T-cell receptor clonotypes, suggesting cellular exchange between the two compartments [7]. Moreover, among patients with NSCLC and BrM receiving camrelizumab-based chemoimmunotherapy, CSF cytokine profiles were associated with intracranial treatment response, further supporting the potential value of CSF-based immune monitoring [8]. Therefore, although CSF cannot fully recapitulate the intracranial TME, it may serve as a minimally invasive liquid-biopsy source for investigating immune features and treatment-related changes in BrM.

Second, previous studies have described stress-responsive and dysfunctional CD8⁺ T-cell states in cancer and BrM. Chu et al. established a pan-cancer T-cell atlas and identified Tstr as a distinct state characterized by increased expression of heat-shock and stress-response genes [5]. In our integrated analysis, CD8⁺ Tstr showed relative enrichment in the BrM and CSF compartments and was associated with proteostasis-related transcriptional programs. These observations are compatible with a stress-responsive phenotype, although differences in sample selection and processing across datasets may also have contributed to the observed tissue distribution. In an inflammatory TME, chronic immune activa-

tion and persistent antigenic exposure may impair T-cell effector function and antitumor immunity [9]. Furthermore, a recent single-cell study of NSCLC BrM identified an HSP70-high T-cell state enriched in intracranial lesions and showed that a higher HSP70-high CD8⁺ T-cell signature was associated with shorter progression-free survival in ICI-treated NSCLC cohorts [6]. Together, these observations support the biological plausibility of an association between proteostasis-related stress programs, dysfunctional T-cell states, and unfavorable immunotherapy outcomes.

Several limitations of this study should be acknowledged. Although cohort-specific clinicopathological analyses were performed, subgroup meta-analyses according to histological subtype, brain-metastatic status, and ICI treatment were not feasible because of the limited availability of comparable datasets. Therefore, future studies should incorporate matched primary tumors, BrM lesions, and CSF specimens from multicenter LUSC cohorts, particularly from patients receiving ICIs. In vitro and in vivo functional experiments, together with relevant animal models, are also required to validate the prevalence, phenotype, and biological function of CD8⁺ Tstr in the CSF and BrM microenvironment and to clarify its association with clinical outcomes.

Conclusion

This case identifies a CD8⁺ Tstr state in pretreatment CSF from a patient with LUSC and BrM. Cross-dataset analyses support its biological relevance, while larger longitudinal cohorts integrating clinical and molecular profiling of CSF are needed for further validation.

Acknowledgements

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Written informed consent was obtained from the patient for the publication and accompanying images.

Disclosure of conflict of interest

None.

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Supplementary Methods

Data acquisition and preprocessing

A 70-year-old man with histologically confirmed lung squamous cell carcinoma (LUSC) and concurrent brain metastasis (BrM) was treated at The First Affiliated Hospital of Anhui Medical University. Pretreatment cerebrospinal fluid (CSF) was obtained by lumbar puncture for single-cell RNA sequencing (scRNA-seq) to characterize the immune-cell composition. Moreover, public scRNA-seq data from tumor-infiltrating PD-1⁺ CD8⁺ T cells isolated from one LUSC BrM lesion (GSM5416355) were obtained from the Gene Expression Omnibus (GEO) database [1]. To enable comparison across tissue compartments, scRNA-seq data from four primary LUSC samples (E-MTAB-13526) were obtained from a previously published study [2]. These datasets were integrated with the CSF sample to compare CD8⁺ T-cell states across primary, metastatic, and CSF compartments.

For external transcriptomic analyses, we included the TCGA-LUSC (n = 542), GSE74777 (n = 107) [3], GSE37745 (n = 66) [4], GSE73403 (n = 69) and GSE30219 (n = 61) [5] cohorts. An immunotherapy dataset (IMvigor210) (n = 298) [6] was used as a cross-cancer exploratory immune-checkpoint-inhibitor dataset to examine associations between T-cell-state signatures and treatment response. Survival analyses were restricted to samples with available survival data. RNA-seq expression values were transformed to $\log_2(\text{TPM} + 1)$ where transcripts-per-million (TPM) data were available. Processed and normalized GEO microarray matrices were used on the scale reported by the original studies. When GSE37745 and GSE73403 were combined, cross-dataset batch effects were adjusted using the ComBat function in the “sva” package.

To investigate the relevance of the stress response T-cell (Tstr) state in BrM, we analyzed paired primary lung tumor and BrM transcriptomic data from lung adenocarcinoma (LUAD) and LUSC cases in the GSE161116 dataset [7]. In addition, a non-small-cell lung cancer (NSCLC) BrM cohort reported by Yang et al. [8] was used to evaluate the associations of Tstr status and clinicopathological factors with overall survival.

CSF sample processing

The CSF sample was filtered through a 40- μm sterile cell strainer and centrifuged at 350 $\times$ g for 5 min. After removal of the supernatant, the cell pellet was resuspended in 1 mL of phosphate-buffered saline (PBS; HyClone). GEXSCOPE[®] red blood cell lysis buffer (RCLB; Singleron Biotechnologies) was added at a cell-suspension-to-RCLB volume ratio of 1:2, followed by incubation at room temperature for 5-8 min to remove residual erythrocytes.

Single-cell library construction and sequencing

The single-cell suspension was adjusted to approximately 1×10^5 cells/mL and loaded onto the GEXSCOPE[®] microfluidic platform. Gene expression and V(D)J libraries were prepared according to the manufacturer's protocol using the GEXSCOPE[®] Single Cell Human V(D)J Kit (Singleron Biotechnologies). Following cell lysis, polyadenylated mRNA was captured by barcoded magnetic beads and labeled with cell- and molecule-specific barcodes. Bead-bound mRNA was reverse-transcribed into complementary DNA (cDNA) and amplified. Gene expression libraries were generated from amplified cDNA, while the remaining cDNA was enriched for immune-receptor sequences and amplified separately to generate V(D)J libraries. Libraries were sequenced on an Illumina NovaSeq 6000 platform using 150-bp paired-end reads.

Preprocessing of single-cell RNA sequencing data

Raw sequencing reads were processed to generate gene expression profiles using CeleScope v1.15.0 (Singleron Biotechnologies) with default parameters. Briefly, barcodes and unique molecular identifiers

Single-cell characterization of cerebrospinal fluid

(UMIs) were extracted from R1 reads and corrected. Adapter sequences and poly(A) tails were trimmed from R2 reads, and the trimmed R2 reads were aligned against the GRCh38 (hg38) reference using STAR (v2.6.1a). Uniquely mapped reads were then assigned to genes with featureCounts (v2.0.1). Successfully assigned reads sharing the same cell barcode, UMI, and gene were collapsed to generate the gene expression matrix for downstream analysis.

Using Seurat v4.3.0, cells with fewer than 200 or more than 5,000 detected genes, or with mitochondrial UMI proportions > 25%, were excluded. The top 2,000 highly variable genes were identified using FindVariableFeatures, followed by data scaling and principal-component analysis (PCA). A shared nearest-neighbor graph was constructed using FindNeighbors, and clusters were identified using FindClusters at a resolution of 0.5. Cells were visualized using t-distributed stochastic neighbor embedding (t-SNE). After quality control and integration, 24,719 cells with 25,925 detected features were retained and grouped into 14 clusters. Batch effects across datasets were corrected using Harmony before neighborhood construction and clustering.

Bioinformatic analysis of scRNA-seq data

Differentially expressed genes (DEGs) for each cell cluster were identified using the FindAllMarkers function in Seurat. The biological functions, exhibited by distinct CD8⁺ T-cell groups, were analyzed and visualized using “ClusterGVis” [9] R package. Tissue enrichment of different CD8⁺ T-cell states was assessed using the ratio of observed to expected (Ro/e) calculated with “STARTRAC” [10] R package.

Immune infiltration and mutation analyses

To investigate the discrepancy of immune profiles, we used “estimate” [11] R package to calculate immune and stromal scores in different infiltration groups. CD8⁺ T-cell-state signature scores were quantified using the single-sample gene set enrichment analysis (ssGSEA) in the TCGA-LUSC, GSE74777, GSE37745, GSE73403, and GSE30219 cohorts. Somatic mutations in the TCGA cohort were examined using the “maftools” [12] R package and the tumor mutation burden (TMB) was calculated for each patient.

Functional enrichment and survival analyses

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were utilized for the exploration of biological functions, with the aid of “clusterProfiler” [13] and “GSVA” [14] R packages. Hallmark gene sets were obtained from the Molecular Signatures Database (MSigDB) using the “msigdb” R package [15]. Utilizing “survival” and “survminer” R packages [16], we compared the survival outcomes of distinct infiltration groups and conducted Kaplan-Meier (KM) analysis. Cohort-specific optimal cut-off values for Tstr-related signature scores were identified using surv_cutpoint, and survival curves were compared using the log-rank test.

Intercellular communication analysis

Intercellular communication was inferred using CellChat (version 1.6.1) [17]. Communication networks were constructed for the primary-tumor and metastatic (BrM + CSF) datasets. CellChat was used to estimate ligand-receptor communication probabilities, aggregate pathway-level signaling, and characterize incoming and outgoing signaling patterns across CD8⁺ T-cell states. Network centrality and pathway-specific ligand-receptor contributions were assessed using netAnalysis_computeCentrality and netAnalysis_contribution, respectively, and selected signaling networks were visualized using netVisual_individual.

Statistical analysis

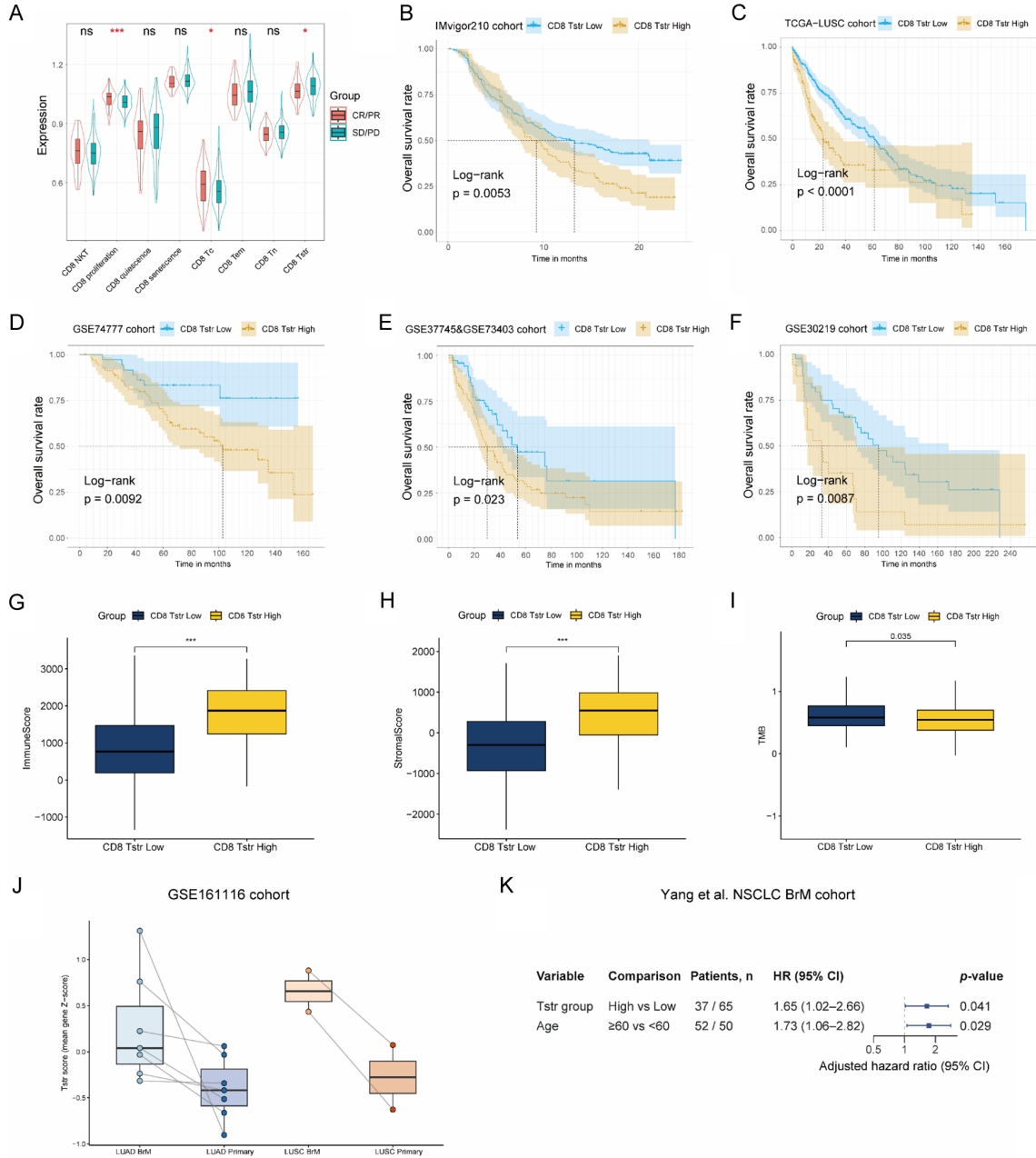
Statistical analyses were performed using R (version 4.3.1) and GraphPad Prism (version 8). Overall survival was evaluated using the KM method, and survival curves were compared using the log-rank test. Continuous variables between two groups were compared using Student’s t-test for normally dis-

tributed data or the Wilcoxon rank-sum test for non-normally distributed data. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

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Single-cell characterization of cerebrospinal fluid



Single-cell characterization of cerebrospinal fluid

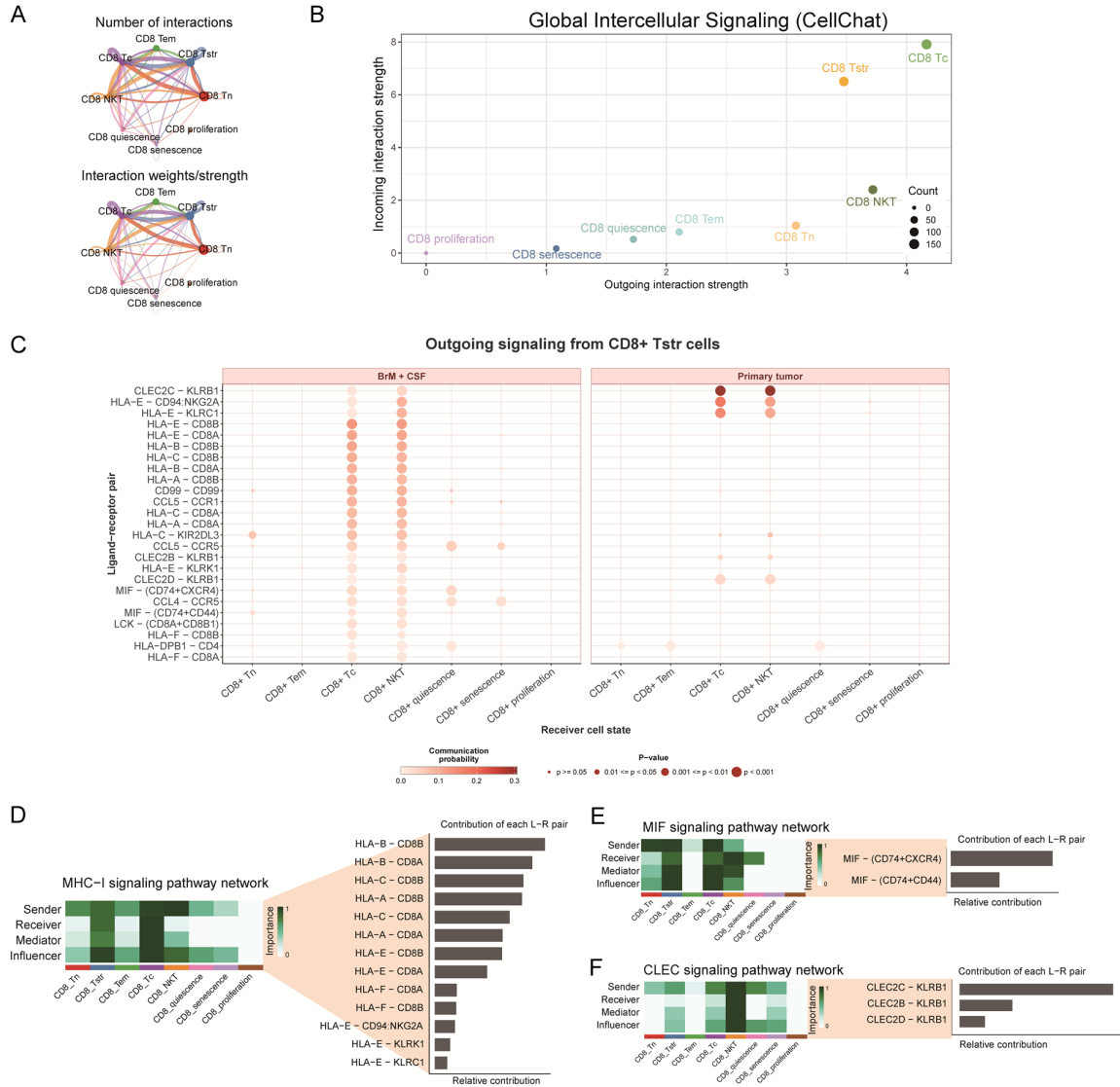


Figure S2. Predicted intercellular communication patterns involving CD8⁺ Tstr across primary and metastatic datasets. A. Network graphs showing the number and strength of predicted interactions among CD8⁺ T-cell states. B. A scatter plot visualizing the interaction strengths of various cells based on their incoming and outgoing signaling patterns. C. Bubble plots showing predicted ligand-receptor interactions in the metastatic (BrM + CSF) and primary-tumor datasets. D-F. Heatmaps demonstrating the biological role of each T-cell category in MHC-I, MIF and CLEC signaling pathways.

Single-cell characterization of cerebrospinal fluid

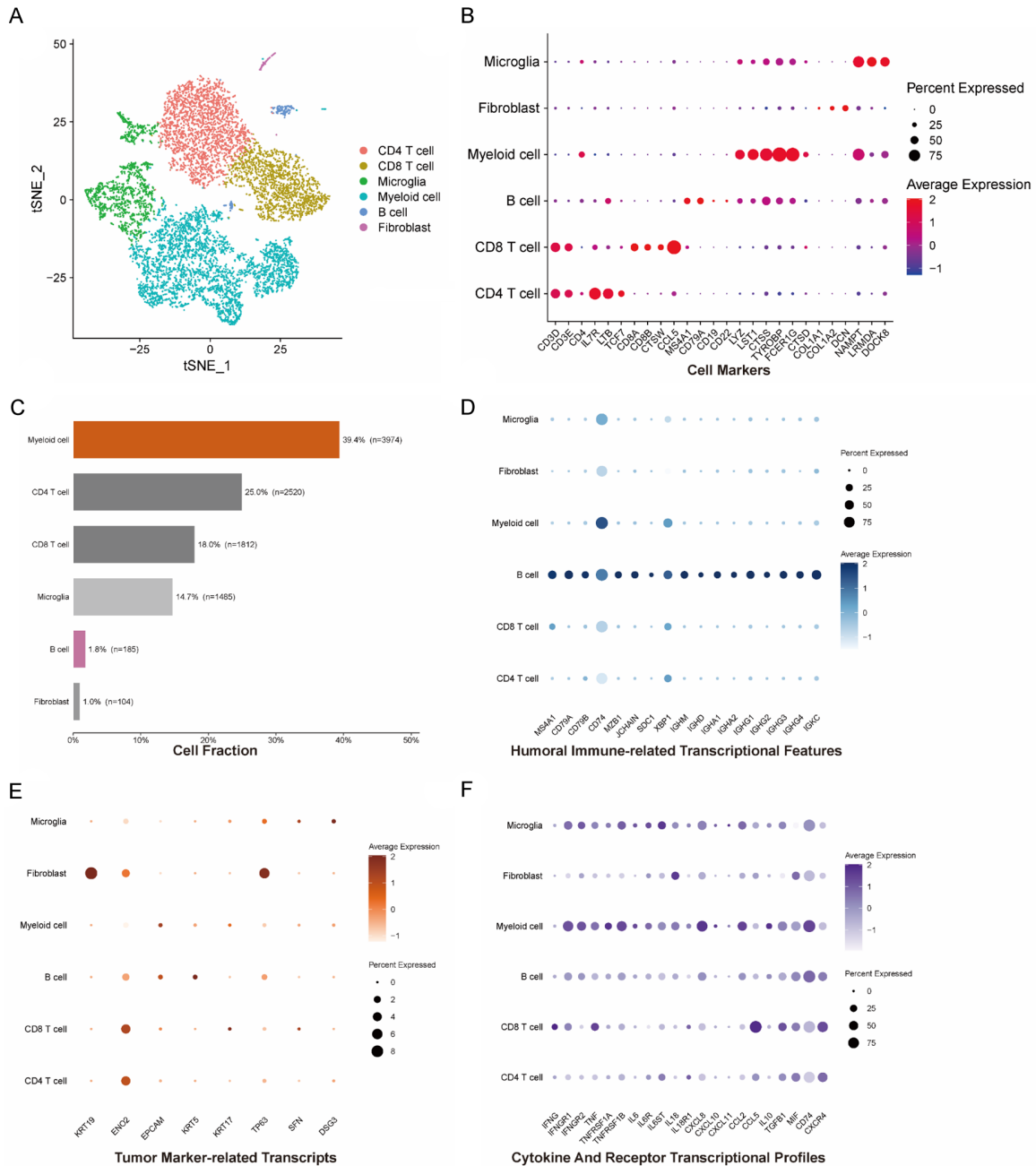


Figure S3. Patient-specific molecular characterization of the pretreatment cerebrospinal fluid (CSF) microenvironment. A. t-SNE visualization of major cell populations identified in the pretreatment CSF scRNA-seq dataset. B. Dot plot showing representative canonical markers supporting cell-type annotation. C. Relative abundance and cell number of the major CSF cellular populations. D. Expression of humoral immune-related transcriptional features, including canonical B-cell markers and immunoglobulin-associated transcripts. E. Expression of tumor marker-related transcripts, including KRT19, ENO2, EPCAM, KRT5, KRT17, TP63, SFN, and DSG3. F. Cytokine- and receptor-related transcriptional profiles across CSF cell populations, highlighting the IFN- γ , TNF- α , and IL-6 signaling axes together with selected chemokines and inflammatory mediators.

Single-cell characterization of cerebrospinal fluid

Table S1. Representative studies related to stress response T-cell and cerebrospinal fluid-based profiling of brain metastasis

Study	Study specimens	Main findings	Relevance to the present study
Immune cell profiling of the cerebrospinal fluid enables the characterization of the brain metastasis microenvironment	scRNA-seq of matched BrM and CSF samples	Immune cell profile of CSF can facilitate the characterization of the immune TME in BrM lesions and longitudinally monitor the evolution of the cancer immune response	CSF profiling reflected the degree of CD8 ⁺ T-cell infiltration in BrM, and identical TCR clonotypes were similarly detected in CSF and intracranial lesions
Cerebrospinal fluid immunological cytokines predict intracranial tumor response to immunotherapy in non-small cell lung cancer patients with brain metastases	Paired CSF and plasma samples from patients with NSCLC BrM receiving camrelizumab-based chemoimmunotherapy	A CSF cytokine model incorporating TIE2, IL-18, LAMP3, PDCD1 and IL-12 was associated with intracranial response	Support the biomarker potential and predictive efficacy of CSF
Pan-cancer T cell atlas links a cellular stress response state to immunotherapy resistance	Pan-cancer atlas comprising 308,048 T-cell transcripts from multiple cancer types	Defined the heat-shock-gene-enriched Tstr state and the expression of Tstr-related signature increased in non-responders	Provide the foundational definition of Tstr and supports an association with immunotherapy resistance
The immunopeptidome landscape associated with T cell infiltration, inflammation and immune editing in lung cancer	Exploration of primary lung cancers from treatment-naïve patients	CD8 ⁺ T-cell infiltration and inflammation were associated with distinct antigen-presentation landscapes	Support the concept that T-cell infiltration and chronic immune pressure are closely linked to immune editing and heterogeneous antigen presentation in lung cancer
Identification of altered immune landscape at single-cell resolution in NSCLC brain metastasis and its association with poor immune checkpoint inhibitor responses	scRNA-seq of tumor-infiltrating immune cells in immunotherapy cohorts	HSP70-high CD4 ⁺ and CD8 ⁺ T cells were enriched in BrM, and higher HSP70-high CD8 ⁺ T-cell signature scores were associated with shorter PFS in immunotherapy cohorts	The external evidence supporting Tstr cells in NSCLC BrM

Abbreviations: BrM, brain metastasis; CSF, cerebrospinal fluid; HSP70, heat shock protein 70; ICI, immune checkpoint inhibitor; NSCLC, non-small cell lung cancer; PFS, progression-free survival; scRNA-seq, single-cell RNA sequencing; TCR, T-cell receptor; TME, tumor microenvironment; Tstr, stress response T cell.