



Integrating single-cell and spatial transcriptomics to decipher the neuro-immune microenvironment of breast cancer brain metastasis: a key regulatory axis, prognostic model, and drug discovery

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Background: Breast cancer brain metastasis (BCBM) is a lethal complication of advanced disease, facilitated by a specialized neuro-immune microenvironment, whereas its molecular regulators and spatial dynamics remain poorly understood. This study aimed to resolve cellular heterogeneity and intercellular signaling networks crosstalk in BCBM microenvironment by using integrated single-cell RNA sequencing (scRNA-seq) single-cell and spatial transcriptomics to identify key regulators, build a prognostic model, and computationally screen for potential therapies.

Methods: We analyzed public scRNA-seq and 10× Visium spatial transcriptomics datasets. Cell landscapes were resolved with Seurat, communication networks were inferred with CellChat, and spatial mapping was performed with Tangram. A prognostic risk model was developed from bulk RNA sequencing (RNA-seq) data from The Cancer Genome Atlas-Breast Cancer (TCGA-BRCA), the Gene Expression Omnibus (GEO) datasets using Least Absolute Shrinkage and Selection Operator-Cox (LASSO-Cox) regression and validated in an independent cohort. Potential small-molecule inhibitors were screened by molecular docking (AutoDock Vina) against AlphaFold-predicted target structures.

Results: The BCBM microenvironment exhibited high heterogeneity, with macrophages serving as the central signaling hub. A novel regulatory axis, interleukin-18 and myosin light chain kinase (*IL-18/MYLK*), was found to be specifically upregulated and co-localized at the tumor-brain interface. A risk-score model based on the *IL-18/MYLK* axis demonstrated robust prognostic value in both training and validation cohorts. The model further revealed that the upregulation of *IL-18* and *MYLK* was associated with better overall survival. Virtual screening identified the MEK inhibitor, *PD98059*, as a high-affinity dual inhibitor of *IL-18* and *MYLK*.

Conclusions: This study delineates the neuro-immune landscape of BCBM progression and proposes the *IL-18/MYLK* axis as a critical driver of neuroinflammation and cytoskeletal remodeling. The prognostic model and the candidate compound *PD98059* provide avenues for novel targeted therapeutic strategies.

Keywords: Breast cancer brain metastasis (BCBM); single-cell RNA sequencing (scRNA-seq); spatial transcriptomics; interleukin-18 (*IL-18*); myosin light chain kinase (*MYLK*)

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Introduction

Breast cancer brain metastasis (BCBM) occurs in 20–40% of advanced breast cancer patients and confers a poor prognosis (1-3). The brain presents a unique metastatic niche defined by the blood-brain barrier (BBB), specialized immune populations such as microglia, and tightly regulated neurovascular interactions (4-6). To colonize this microenvironment, tumor cells must acquire adaptive traits, including epithelial-mesenchymal plasticity, metabolic remodeling, and immune evasion (7). Current therapeutic strategies for BCBM—including surgery, radiotherapy, and systemic agents—remain largely palliative, with limited efficacy of immune checkpoint blockade attributed to poor T-cell infiltration and spatially restricted immune exclusion (8,9). Accumulating evidence underscores the neuro-

immune microenvironment as a critical regulator of BCBM progression (10,11). However, the cellular heterogeneity, molecular mediators, and spatial architecture governing these interactions remain poorly defined (12). In particular, the intersection between neuroinflammatory signaling and tumor biomechanical adaptation during brain metastasis is largely unexplored (13).

Traditional techniques, such as bulk sequencing, are limited by resolution and were unable to fully capture the heterogeneity and spatial context of this microenvironment (14). Recent advances in single-cell and spatial transcriptomics now provide unprecedented opportunities to overcome these limitations (15-17). Therefore, this study investigated interleukin-18 (*IL-18*), a neuro-immune regulatory factor (18), which has not been systematically studied in BCBM progression. Emerging evidence suggests that *IL-18* exerts context-dependent effects on tumor immune evasion, particularly through the *IL-18/IL-18BP* balance (19). This study integrates multi-omics data to dissect the BCBM microenvironment with high-resolution transcriptomics, elucidate the role of the *IL-18*/myosin light chain kinase (*MYLK*) axis, and evaluate its prognostic and therapeutic relevance (20). We present this article in accordance with the TRIPOD reporting checklist (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-2025-1-2898/rc>).

Highlight box

Key findings

- Single-cell and spatial transcriptomics reveal that macrophages act as the central signaling hub in the breast cancer brain metastasis (BCBM) microenvironment.
- Interleukin-18 (*IL-18*) and myosin light chain kinase (*MYLK*) are spatially co-localized at the tumor-brain interface, defining a novel inflammation-mechanics coupling axis that promotes metastatic invasion.
- A prognostic risk model incorporating *IL-18* and *MYLK* robustly stratifies patient survival and reflects neuro-immune microenvironmental states.
- *PD98059*, a MEK inhibitor, is identified via molecular docking as a potential dual-target inhibitor of *IL-18* and *MYLK*, offering a repurposing opportunity.

What is known and what is new?

- BCBM involves a unique neuro-immune microenvironment with immunosuppressive features and limited therapeutic options.
- This study identifies the *IL-18/MYLK* axis as a spatially organized driver of biomechanical inflammation, and computationally repurposes *PD98059* as a dual-target candidate.

What is the implication, and what should change now?

- Targeting *IL-18* and *MYLK* may simultaneously suppress tumor invasion and immune exclusion.
- Future therapies should consider microenvironment-normalizing agents like *PD98059* in combination with immunotherapies to enhance T-cell infiltration and improve outcomes.

Methods

Data sources

All data were obtained from public databases. The Single-cell RNA sequencing (scRNA-seq) dataset from Gene Expression Omnibus (GEO) of GSE268662 includes tumor and adjacent normal tissues from 9 breast cancer patients. The 10× Visium spatial transcriptomics dataset GSE240212 contains 2 slices of *MDA-MB-231* BCBM samples. Bulk RNA-seq (RNA-seq) data and clinical information were sourced from 4. The Cancer Genome Atlas-Breast Cancer dataset (TCGA-BRCA) (n=1,098) and GEO datasets GSE52604 (n=55) and GSE100534 (n=11 paired samples). All data were retrieved between August 2024 and March 2025. Datasets are summarized in *Table 1*. The study was conducted in accordance with the Declaration of Helsinki

Table 1 Overview of publicly available datasets used in this study

Database	Accession ID	Data type	Sample description	Primary use
GEO	GSE268662	Single-cell RNA-seq	9 breast cancer patients (tumor/adjacent normal)	Cellular heterogeneity and IL-18 expression analysis
	GSE240212	10× Visium spatial transcriptomics	2 slices of MDA-MB-231 BCBM samples	Spatial expression pattern and hotspot analysis
	GSE52604	Bulk RNA-seq	55 BCBM and control tissue samples	Differential gene screening
TCGA	TCGA-BRCA	Bulk RNA-seq + Clinical data	1,098 breast cancer cases (including BCBM subgroup)	Survival analysis and model construction
GEO	GSE100534	Bulk RNA-seq	11 paired BCBM samples with complete survival information	External independent validation

BCBM, breast cancer brain metastasis; GEO, Gene Expression Omnibus; IL-18, interleukin-18; TCGA-BRCA, The Cancer Genome Atlas Breast Cancer dataset.

and its subsequent amendments.

Data processing and analysis

Raw sequencing data (GSE268662) were aligned to GRCh38 using CellRanger (v7.1.0). Downstream analysis was performed in R (v4.3.2) with Seurat (v5.0.1). Quality control removed cells with nFeature_RNA <200 or >6,000 and percent.mt >15%. Data were normalized with SCTransform. Batch effects were corrected with Harmony (v0.1.1). Principal component analysis (PCA) was performed on integrated data, and clustering used the top 30 principal components (PCs) (FindNeighbors/FindClusters, resolution =0.8). Uniform Manifold Approximation and Projection (UMAP) was used for visualization.

Cell type annotation and cell-cell communication analysis

Cell subpopulations were annotated with SingleR (v2.4.1) using the Human Cell Atlas, followed by manual correction using canonical markers: epithelial/tumor cells (*EPCAM*, *KRT8*, *KRT18*), macrophages (*CD68*, *CD163*), microglia (*TMEM119*, *P2RY12*), T cells (*CD3D*, *CD8A*, *CD4*), B cells (*CD79A*, *MS4A1*), endothelial cells (*PECAM1*, *VWF*), and fibroblasts (*DCN*, *COL1A1*). Cell-cell communication was analyzed with CellChat (v2.1.2), focusing on neuro-immune relevant pathways.

Integration and spatial analysis of spatial transcriptomic data

Spatial data (GSE240212) were processed with Space

Ranger (v2.0.0). Cellular composition was deconvolved using Tangram to map annotated scRNA-seq data onto spatial slices. Gene expression patterns (e.g., *IL-18*, *MYLK*) were visualized from normalized counts. Spatial hotspots were identified with the SpatialDE package.

Analysis of bulk RNA-Seq data and construction of the prognostic model

Bulk RNA-seq data were converted to transcripts per million (TPM) and $\log_2(\text{TPM} + 1)$ transformed. To identify robust brain metastasis-associated genes, differential expression analysis between BCBM and control tissues was performed separately in four independent cohorts: GSE268662, GSE240212, GSE52604, and TCGA-BRCA, using the limma package (v3.58.1) with thresholds of $|\log_2\text{FC}| > 1$ and false discovery rate (FDR) <0.05. Genes consistently differentially expressed across these cohorts were considered core candidates for downstream analysis. To identify survival-associated genes among these candidates, we first conducted univariate Cox regression analysis ($P < 0.05$) within the TCGA-BRCA cohort, which contains comprehensive clinical follow-up data. Subsequently, to address multicollinearity and avoid overfitting, we applied Least Absolute Shrinkage and Selection Operator-Cox (LASSO-Cox) regression (glmnet v4.1.8) with 10-fold cross-validation. This procedure selected the optimal combination of prognostic genes and their corresponding regression coefficients (β). A risk-score formula was then constructed as a linear combination of these selected genes' expression values weighted by their LASSO coefficients: Risk score = $\sum (\beta_i \times \text{Expi})$, where Expi represents the normalized expression

level of gene *i*. In our final model, the selected genes included *IL-18* and *MYLK*, among others. Based on this formula, a risk score was calculated for each patient in the TCGA-BRCA cohort, and patients were stratified into high- and low-risk groups using the median risk score as the cut-off point. The model's performance was evaluated by Kaplan-Meier survival analysis, hazard ratio (HR) estimation, and time-dependent receiver operating characteristic (ROC) curve analysis using the 'survival' and 'timeROC' packages. Finally, the independent predictive value of this risk model was rigorously validated in an external cohort, GSE100534, which includes valuable paired brain metastasis samples.

Molecular docking

Structures of human *IL-18* (UniProt: Q14116) and *MYLK* (UniProt: Q15746) were obtained from the AlphaFold database. Ligand structures (*PD98059*, *ethanol*) were downloaded from PubChem and prepared with AutoDockTools. Docking was performed with AutoDock Vina (v1.2.3) using a blind-docking strategy (exhaustiveness =128). Binding free energies (ΔG , kcal/mol) were extracted from output. Binding poses were visualized with PyMOL (v2.5.0) to identify key interactions.

Statistical analysis

All analyses were conducted in R (v4.3.2). Group differences were assessed with two-tailed Wilcoxon rank-sum tests. Survival differences were evaluated with the log-rank test. Multiple testing was controlled by the Benjamini-Hochberg method. $P < 0.05$ or FDR < 0.05 was considered significant.

Results

Single-cell atlas and intercellular communication network of the BCBM neuro-immune microenvironment

To systematically dissect the cellular ecosystem of BCBM, we performed scRNA-seq analysis on nine BCBM samples covering major molecular subtypes (HER2⁺, Luminal B, triple-negative). After stringent quality control and batch effect correction using Harmony, unsupervised clustering identified seven major cell lineages: tumor cells, macrophages, microglia, T cells, B cells, endothelial cells, and fibroblasts (Figure 1A). Tumor cells exhibited pronounced intra-lineage heterogeneity, comprising proliferative (*MKI67*⁺/*TOP2A*⁺) and invasive epithelial-

mesenchymal transition (EMT)-like (*VIM*⁺/*FN1*⁺) subpopulations, suggesting functional diversification during brain colonization.

Immune cells constituted the most abundant non-tumor compartment, challenging the traditional view of the brain as an immune-privileged site. Notably, CD8⁺ T cells displayed high expression of exhaustion markers (*PD-1*, *CTLA4*, *LAG3*) and low levels of effector molecules (*GZMB*, *PRF1*), indicating functional impairment. Myeloid cells emerged as the dominant immune population, further subdivided into peripherally derived macrophages (*CD68*⁺/*CD163*⁺/*MRC1*⁺) exhibiting M2-like immunosuppressive polarization, and brain-resident microglia (*TMEM119*⁺/*P2RY12*⁺/*CX3CR1*⁺). Spatial mapping using Seurat integration revealed a distinctive "immune-excluded" architecture: immune cells, particularly macrophages, surrounded tumor nests, while the tumor core remained largely immune-sparse (Figure 1B).

Intercellular communication analysis using CellChat identified macrophages as the central signaling hub, exhibiting the highest number and strength of interactions with other cell types (Figure 1C, 1D). Key ligand-receptor pairs included *CXCL12*-*CXCR4*, mediating tumor cell chemoattraction and survival, and *VEGFA*-*VEGFR2*, driving pathological angiogenesis and BBB disruption (Figure 1E). Additional myeloid-tumor interactions, such as *ANGPT2* – (*ITGA5* + *ITGB1*) and *ANXA1*-*FPR1*, suggested roles in macrophage recruitment, M2 polarization, and phagocytosis evasion. These findings establish macrophages as master regulators of the BCBM neuro-immune ecosystem and reveal a pro-metastatic signaling network centered on myeloid-tumor crosstalk—providing the cellular foundation for subsequent exploration of inflammation-driven biomechanical remodeling.

Spatial co-localization of IL-18 and MYLK at the tumor-brain interface: linking neuroinflammation to biomechanical remodeling

Building on the identification of macrophages as the central signaling hub, we next investigated how myeloid-derived inflammatory signals might translate into biomechanical changes that facilitate brain colonization. To overcome the spatial resolution limitations of scRNA-seq, we integrated single-cell transcriptomic profiles with spatial transcriptomics data using Tangram mapping, reconstructing the tissue-level distribution of nine distinct cell subpopulations (Figure 2A, 2B). This integrative analysis confirmed the immune-excluded architecture and further

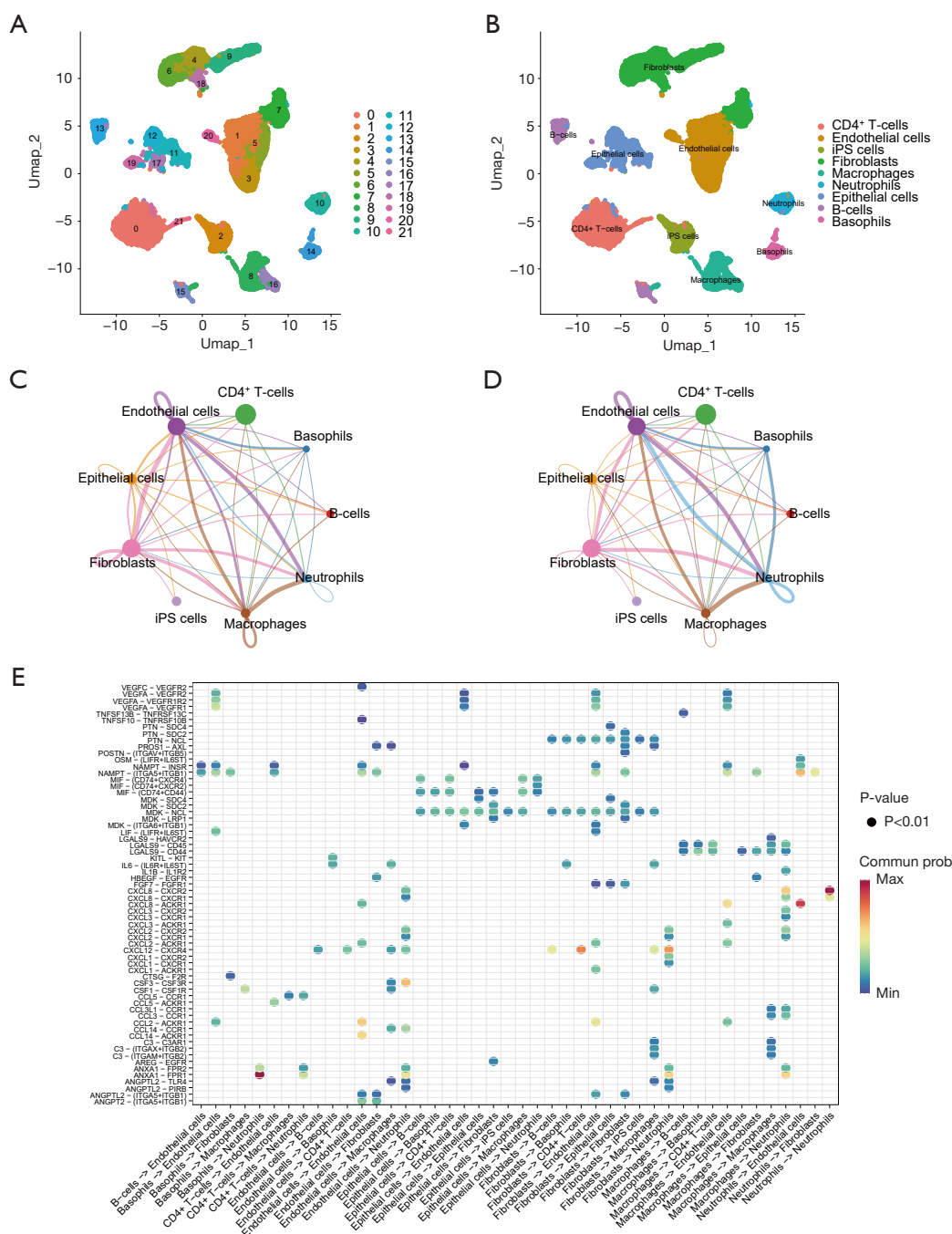


Figure 1 Single-cell atlas and cell-cell communication network in the breast cancer brain metastasis microenvironment. (A) UMAP visualization of single-cell RNA sequencing data from nine breast cancer samples. Each dot represents a single cell, colored by distinct cell clusters. (B) Spatial distribution of major cell types within a representative breast cancer sample. Colors indicate key cellular populations, including epithelial/tumor cells, immune cells (macrophages, T cells, B cells), and stromal cells (endothelial cells, fibroblasts). (C) Network of inferred ligand-receptor interaction numbers between cell clusters, analyzed using CellChat. Edge width reflects the frequency of interactions between cell types. (D) Network of inferred ligand-receptor interaction strengths. Edge color intensity represents the interaction probability. (E) Analysis of key neuro-immune-related ligand-receptor pairs. The bubble plot displays interaction strength and statistical significance for selected pairs, including ANGPT2-(ITGA5+ITGB1), ANXA1-FPR1, CCL5-CCR1, CXCL12-CXCR4, and VEGFA-VEGFR2. UMAP, Uniform Manifold Approximation and Projection.

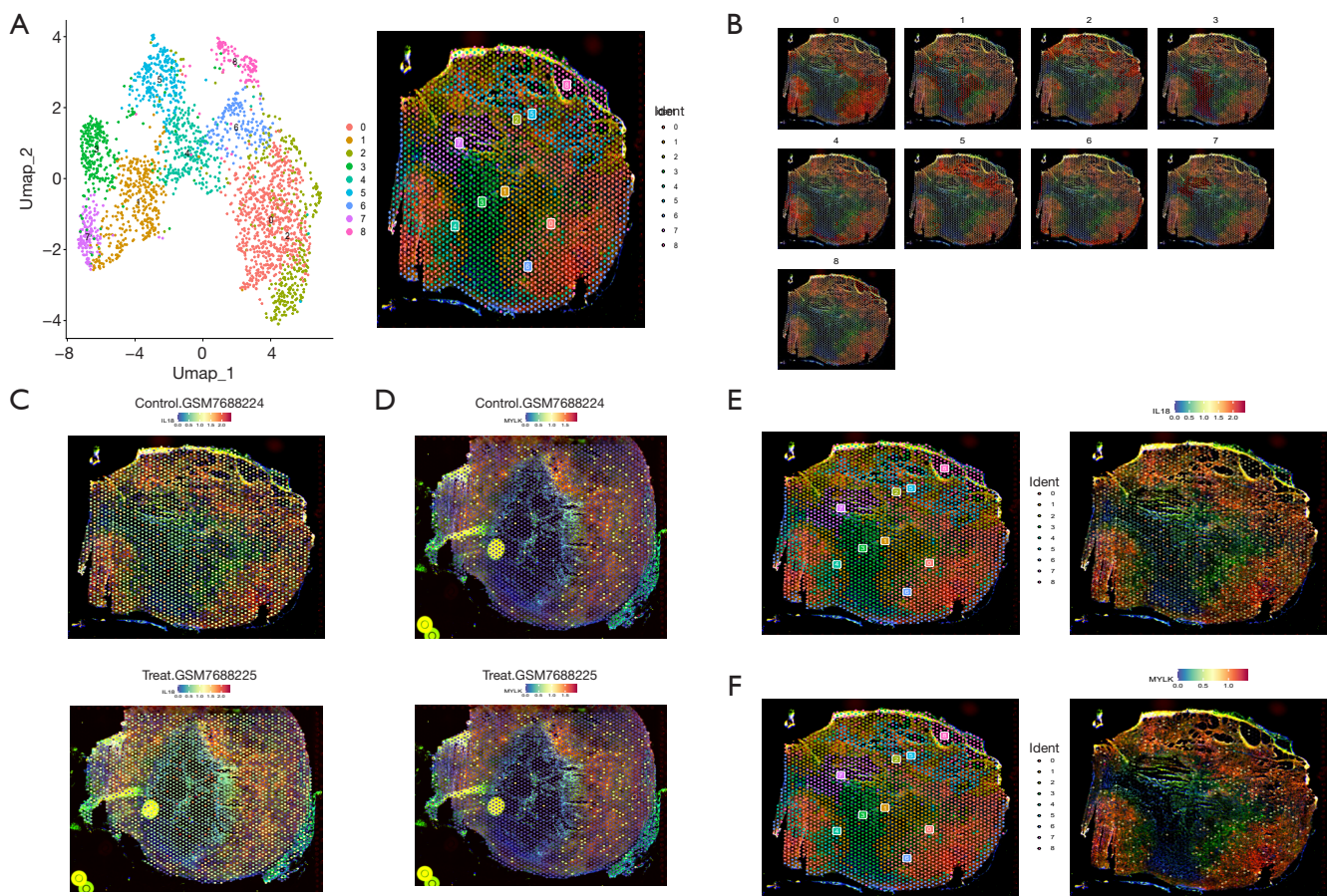


Figure 2 Spatial co-localization and cellular source of interleukin-18 and myosin light chain kinase in the breast cancer brain metastasis microenvironment. (A) UMAP plot of integrated single-cell RNA sequencing and spatial transcriptomic data (Seurat), revealing nine distinct cell clusters (0–8). (B) Distribution of each individual cell cluster within the UMAP embedding. (C) Spatial expression patterns of interleukin-18 in normal brain tissue (top, GSM7688224) and a BCBM sample (bottom, GSM7688225). Color scale indicates expression levels. (D) Spatial expression patterns of myosin light chain kinase in normal brain tissue (top, GSM7688224) and the breast cancer brain metastasis sample (bottom, GSM7688225). (E) Spatial expression patterns of interleukin-18 across distinct cell subpopulations in the breast cancer brain metastasis sample. (F) Spatial expression distribution of myosin light chain kinase across distinct cell subpopulations in the breast cancer brain metastasis sample. BCBM, breast cancer brain metastasis; UMAP, Uniform Manifold Approximation and Projection.

localized macrophages to the invasive tumor margin, where they formed physical niches adjacent to tumor cells—providing the structural basis for paracrine signaling.

Focusing on candidate genes implicated in neuro-immune crosstalk and cytoskeletal regulation, we examined the spatial expression patterns of *IL-18* and *MYLK*. Compared to normal brain tissue, BCBM samples exhibited marked upregulation of both genes, with expression hotspots concentrated at the tumor-brain interface and perivascular regions (Figure 2C,2D). Spatial correlation analysis revealed significant co-localization of *IL-18* and *MYLK* transcripts across tissue spots. Cell-type mapping

further demonstrated that *IL-18* was primarily expressed by tumor-associated macrophages and a subset of stress-responsive tumor cells, whereas *MYLK* was enriched in invasive tumor cells and endothelial cells (Figure 2E,2F).

This spatially coordinated expression pattern—macrophage/tumor-derived *IL-18* adjacent to *MYLK*⁺ tumor/endothelial cells—strongly suggests a paracrine signaling axis linking neuroinflammation to biomechanical remodeling. Specifically, *IL-18* secreted by macrophages may activate downstream *NF-κB* signaling in neighboring cells, inducing *MYLK* expression and subsequent cytoskeletal contraction. This mechanism could

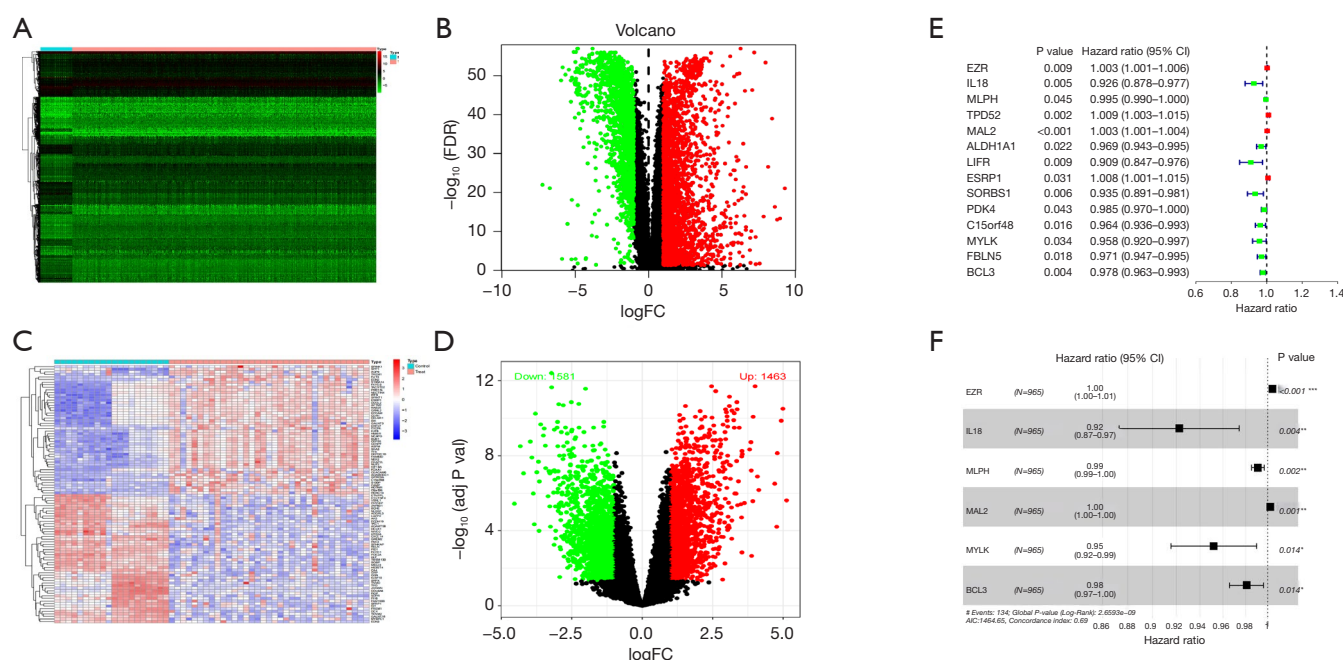


Figure 3 Screening of differentially expressed genes and their prognostic value based on Multi-Cohort data. (A) Heatmap of differentially expressed genes between breast cancer tissues and adjacent normal tissues in The Cancer Genome Atlas Breast Cancer dataset cohort. Rows represent genes, columns represent samples, and color indicates standardized expression levels (red for high expression, green for low expression). Genes showing the most significant expression differences are displayed. (B) Volcano plot of differentially expressed genes in The Cancer Genome Atlas Breast Cancer dataset cohort. Each point represents a gene; the x-axis shows the $\log_2$ fold change, and the y-axis shows the negative logarithm of the adjusted P value. Red points denote significantly up-regulated genes, green points denote significantly down-regulated genes, and black points represent non-significant genes. (C) Heatmap of differentially expressed genes between control and treatment groups in the GSE52604 cohort. Rows indicate genes, columns indicate samples, and standardized expression levels are color-coded (red for high expression, blue for low expression). The most significantly altered genes are shown. (D) Volcano plot of differential expression analysis in the GSE52604 cohort. Each dot corresponds to one gene, with the x-axis representing $\log_2$ fold change and the y-axis representing the negative logarithm of the adjusted P value. Red dots indicate significantly up-regulated genes (n=1,463), green dots indicate significantly down-regulated genes (n=1,581), and black dots represent non-significant genes. (E) Forest plot of univariate Cox regression analysis for key candidate genes in relation to overall survival of breast cancer patients. A hazard ratio >1 suggests that high expression of the gene is associated with poorer prognosis. (F) Forest plot of the multivariate Cox regression model. After adjusting for other clinicopathological factors, genes such as interleukin-18 and myosin light chain kinase were identified as independent prognostic factors. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. AIC, Akaike Information Criterion; CI, confidence interval; FC, fold change; FDR, false discovery rate.

simultaneously enhance tumor cell amoeboid migration through dense brain parenchyma and disrupt endothelial tight junctions, promoting BBB leakage and peritumoral edema—two hallmarks of BCBM progression that critically depend on the neuro-immune microenvironment.

IL-18 and MYLK serve as independent prognostic factors: clinical relevance of the neuro-immune axis

Having established the cellular and spatial basis of the *IL-18/MYLK* axis within the BCBM microenvironment, we

next sought to determine whether this axis carries clinical significance for patient outcomes. To translate our findings into clinical relevance, we extended our analysis to large bulk RNA-seq cohorts. In the TCGA-BRCA cohort, both *IL-18* and *MYLK* were significantly upregulated in breast cancer tissues compared to normal breast (Figure 3A,3B). More importantly, in the paired primary-BCBM cohort (GSE52604)—which includes 35 brain metastases, 10 normal brain, and 10 normal breast samples—both genes were specifically elevated in brain metastases relative to matched primary tumors (Figure 3C,3D), indicating their

selective involvement in the metastatic process rather than tumorigenesis *per se*.

Univariate Cox regression analysis revealed that high expression of *IL-18* and *MYLK* correlated with improved overall survival in breast cancer patients—a finding that initially appeared paradoxical given their pro-metastatic roles in local microenvironments. Multivariate Cox analysis, adjusting for age, stage, and receptor status, confirmed both genes as independent protective factors (Figure 3E,3F). This “function–prognosis paradox” suggests a context-dependent duality: while *IL-18* and *MYLK* contribute to local metastatic colonization within the neuro-immune microenvironment, their systemic upregulation may reflect a compensatory host defense response—linking microenvironmental dynamics to overall patient prognosis.

Construction and validation of an *IL-18/MYLK*-based risk prediction model: capturing microenvironmental states

Based on the recognition that *IL-18* and *MYLK* expression reflects distinct states of the neuro-immune microenvironment, we hypothesized that a composite signature incorporating these genes could stratify patients by microenvironmental context and, consequently, by clinical risk. Using LASSO-Cox regression, we constructed a risk score model incorporating *IL-18*, *MYLK*, and their co-expressed signatures. The final model comprised six genes:

Risk score = $(0.0041 \times EZR) + (-0.0809 \times IL-18) + (-0.0090 \times MLPH) + (-0.3261 \times MAL2) + (-0.0492 \times MYLK) + (-0.0194 \times BCL3)$, where values are $\log_2(\text{TPM} + 1)$ transformed.

Notably, the negative coefficients for *IL-18* and *MYLK* indicate that higher expression of these microenvironmental markers lowers the risk score—consistent with the interpretation that tumors eliciting robust immune and structural responses (reflected in *IL-18/MYLK* upregulation) are associated with better outcomes. In the TCGA-BRCA training cohort, the model demonstrated good predictive performance with a AUC of 0.729 (Figure 4A), excellent calibration (mean absolute error = 0.024; Figure 4B), and positive clinical net benefit across threshold probabilities (Figure 4C). Strikingly, when applied to the independent GSE100534 validation cohort, the model achieved near-perfect discrimination (AUC = 0.987; Figure 4D–4G), underscoring its robustness.

Kaplan-Meier survival analysis further confirmed that high *IL-18* expression correlated with prolonged survival

($P=0.008$; Figure 4H), as did high *MYLK* expression ($P=0.04$; Figure 4I). This model thus captures the “microenvironmental defense signature”—quantifying the state of neuro-immune activation and structural stabilization—and translates it into a clinically actionable tool for risk stratification.

Virtual screening identifies PD98059 as a potential dual-target inhibitor of the *IL-18/MYLK* axis

Having established the *IL-18/MYLK* axis as a key regulator of the BCBM neuro-immune microenvironment with prognostic significance, we next explored therapeutic strategies to modulate this axis. Using high-resolution structures from the AlphaFold database and AutoDock Vina for molecular docking, we screened a library of compounds against *IL-18* and *MYLK*. *Ethanol* served as a negative control, showing weak, non-specific binding to both targets ($\Delta G = -2.5$ and -3.0 kcal/mol, respectively; Figure 5A,5B).

Among screened compounds, *PD98059*—a well-known MEK inhibitor—emerged as a top candidate with high-affinity binding to both targets. *PD98059* bound *IL-18* with a binding energy of -6.9 kcal/mol at a pocket corresponding to the *IL-18/IL-18Ra* receptor interface, suggesting competitive inhibition of receptor engagement (Figure 5C). Key interacting residues (*Ile116*, *Leu174*, *Arg63*) are known to be critical for *IL-18* biological activity, supporting functional relevance.

Remarkably, *PD98059* bound *MYLK* with even higher affinity ($\Delta G = -8.2$ kcal/mol), deeply embedding into the ATP-binding pocket of the kinase domain (Figure 5D). The compound formed stable hydrogen bonds with catalytic residues (*Lys1493*, *Asp1605*) and established π - π stacking interactions with *Phe1475*, collectively occupying the ATP-binding site and likely inhibiting kinase activity. This dual-target binding profile—blocking upstream inflammatory signaling (*IL-18*) while simultaneously suppressing downstream cytoskeletal effector (*MYLK*)—positions *PD98059* as a promising candidate for disrupting the inflammation-mechanics axis that we have identified as central to the BCBM neuro-immune microenvironment (Table 2).

Discussion

By integrating single-cell and spatial transcriptomics, this study characterizes the neuro-immune microenvironment of BCBM. We identify macrophages as an intercellular

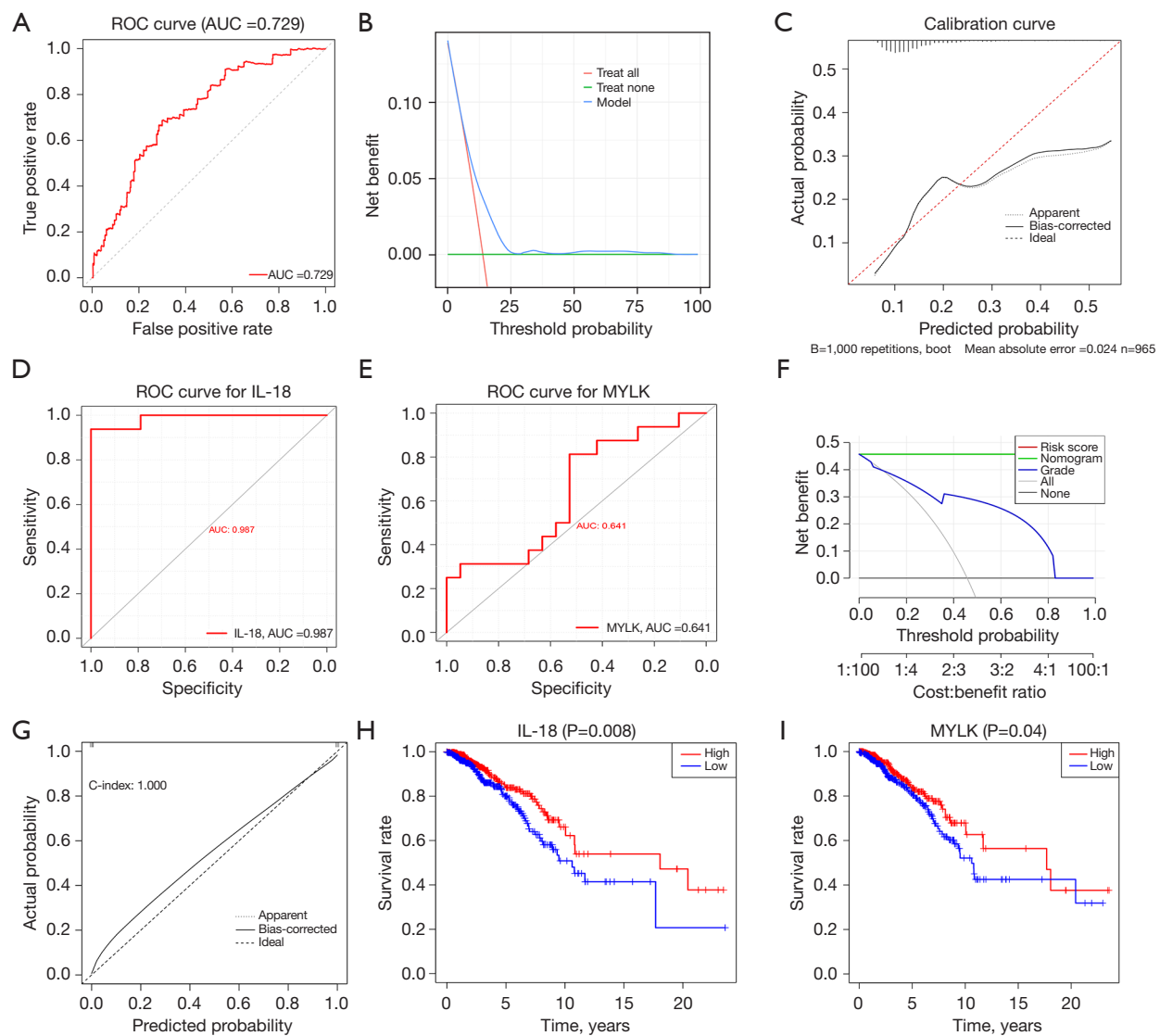


Figure 4 Construction and validation of a breast cancer brain metastasis risk prediction model based on interleukin-18 and myosin light chain kinase. (A) Receiver operating characteristic curve of the combined prediction model constructed by multivariate Cox regression analysis in the training cohort. The area under the curve is 0.729. (B) Calibration curve of the combined prediction model. The predicted risk shows good agreement with the observed risk, with a mean absolute error of 0.024. (C) Decision curve analysis for the combined prediction model. The results indicate that applying this model provides a net clinical benefit across a range of threshold probabilities. (D) Receiver operating characteristic curve for the interleukin-18 gene in the GSE100534 cohort. The area under the curve is 0.987. (E) Receiver operating characteristic curve for the myosin light chain kinase gene in the GSE100534 cohort. The area under the curve is 0.641. (F) Calibration curve of the GSE100534 nomogram after internal validation using the bootstrap method (1,000 resamples). The concordance index is 1.000. (G) Decision curve analysis for the GSE100534 nomogram. (H) Kaplan-Meier survival curves for overall survival based on interleukin-18 expression. Patients were stratified into high- and low-expression groups according to the median expression level (log-rank test, $P=0.008$). (I) Kaplan-Meier survival curves for overall survival based on myosin light chain kinase expression. Patients were stratified into high- and low-expression groups according to the median expression level (log-rank test, $P=0.04$). AUC, area under the curve; ROC, receiver operating characteristic.

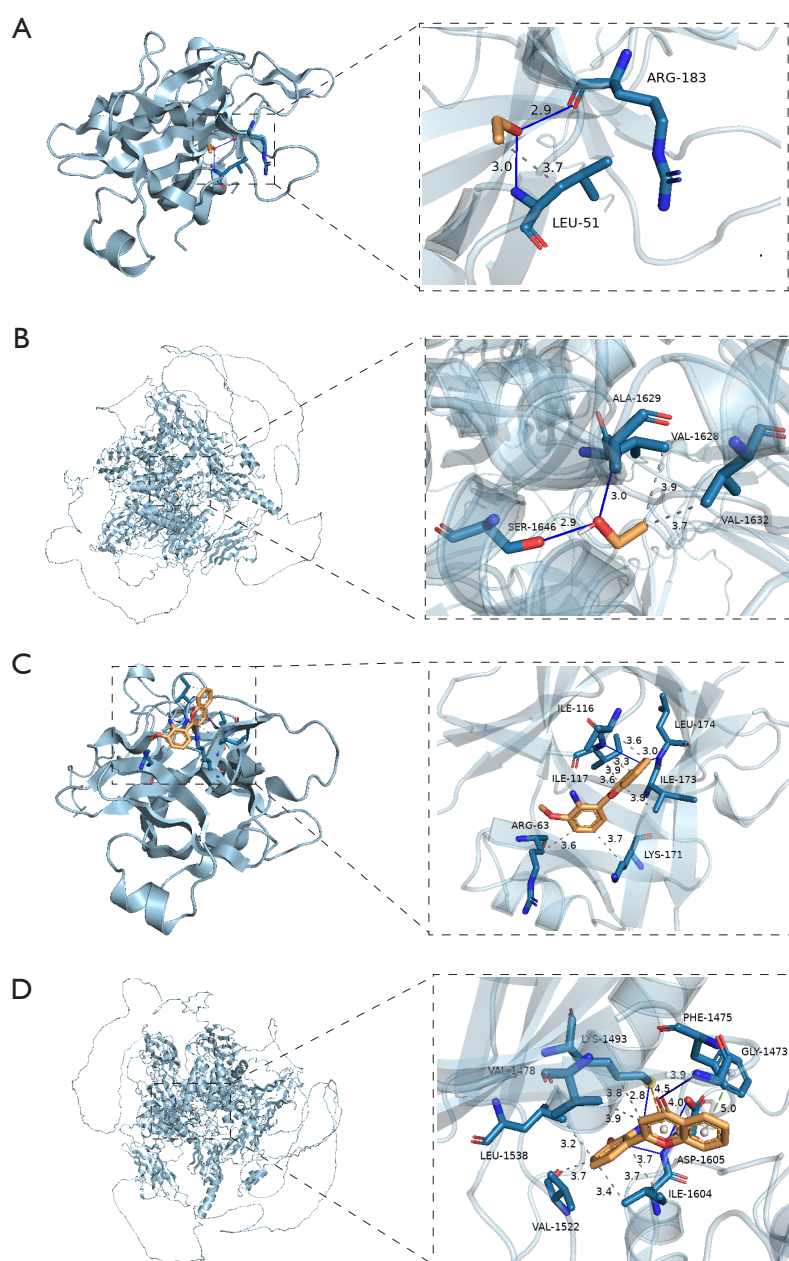


Figure 5 Lead compound screening targeting interleukin-18 and myosin light chain kinase and validation by molecular docking. (A) Molecular docking model of the candidate compound ethanol with the interleukin-18 protein. (Left) Global binding mode. (Right) Detailed view of local interactions (blue solid lines represent hydrogen bonds, black dashed lines represent hydrophobic interactions). The binding free energy is -2.5 kcal/mol. (B) Molecular docking model of the candidate compound ethanol with the myosin light chain kinase protein. (Left) Global binding mode. (Right) Detailed view of local interactions (blue solid lines represent hydrogen bonds, black dashed lines represent hydrophobic interactions). The binding free energy is -3.0 kcal/mol. (C) Molecular docking model of the lead compound PD98059 with the interleukin-18 protein. (Left) Global binding mode. (Right) Detailed view of local interactions, showing a network of hydrogen bonds and hydrophobic interactions formed between PD98059 and residues within the binding pocket. The binding free energy is -6.9 kcal/mol. (D) Molecular docking model of the lead compound PD98059 with the myosin light chain kinase protein. (Left) Global binding mode. (Right) Detailed view of local interactions, showing that PD98059 forms hydrogen bonds, hydrophobic interactions, and π -stacking interactions (green dashed lines) with myosin light chain kinase. The binding free energy is -8.2 kcal/mol.

Table 2 Molecular docking results for representative compounds

Compound	Target	Binding energy (kcal/mol)	Binding pocket	Key interacting residues	Interaction types
Ethanol	IL-18	−2.5	c5	ARG-183, LEU-51	Hydrogen bond, hydrophobic
	MYLK	−3.0	c1	ALA-1629, VAL-1628, SER-1646, VAL-1632	Hydrogen bond, hydrophobic
PD98059	IL-18	−6.9	c4	ILE-116, LEU-174, ILE-117, ARG-63, LYS-171	Hydrogen bond, hydrophobic, π -cation
	MYLK	−8.2	c1	PHE-1475, GLY-1473, LYS-1493, ASP-1605	Hydrogen bond, hydrophobic, π -stacking

PD98059, a specific MEK inhibitor; MYLK, myosin light chain kinase; binding pockets (c1, c4, c5) refer to specific structural pockets in the respective protein models. Interaction types: π -cation, π -stacking are aromatic interactions. IL-18, interleukin-18.

communication signaling hub and propose the *IL-18/MYLK* axis as a key driver of metastasis. These computational findings suggest a pathway linking neuroinflammation with cytoskeletal remodeling in BCBM progression, whereas further experimental validation is still needed (21).

The BCBM neuro-immune microenvironment: from cellular architecture to functional axis

This study provides a spatially resolved, multi-dimensional dissection of the BCBM neuro-immune microenvironment, tracing a logical path from cellular composition to functional axis to clinical translation. Our single-cell atlas first establishes that BCBM is not an immune desert but rather an “immune-excluded” ecosystem, characterized by abundant myeloid infiltration juxtaposed with T cell exclusion from tumor nests (22–24). Macrophages—both peripherally derived and brain-resident microglia—emerge as the central signaling hub, orchestrating tumor–stroma crosstalk through multiple pro-metastatic axes (25–27).

Building on this cellular foundation, spatial transcriptomics reveals that the *IL-18/MYLK* axis operates at the critical interface between inflammation and mechanics (28). The co-localization of *IL-18* (expressed by macrophages/stress-responsive tumor cells) and *MYLK* (enriched in invasive tumor cells/endothelial cells) at the invasive tumor front represents a key architectural feature—a spatially organized circuit linking neuroinflammation to biomechanical remodeling (29,30). This finding transforms our understanding of BCBM progression: rather than viewing inflammation and mechanical invasion as separate processes, they emerge as coupled phenomena orchestrated

within the microenvironment (31).

The IL-18/MYLK axis and the “function-prognosis paradox”: context-dependent roles in the neuro-immune ecosystem

Perhaps the most intriguing insight emerging from this integrated analysis is the recognition that the same molecular axis can exert distinct effects depending on microenvironmental context—a principle we term the “function–prognosis paradox” (32). Our spatial and cellular data clearly implicate the *IL-18/MYLK* axis in promoting local metastatic colonization via *EMT* induction, cytoskeletal remodeling, and barrier disruption (33). Yet, paradoxically, its systemic upregulation correlates with favorable outcomes (34,35).

This paradox is resolved by considering the dual roles of neuro-immune interactions (36,37): locally, within the tumor microenvironment, *IL-18* derived from tumor-associated macrophages drives pro-metastatic inflammation, activating downstream pathways that induce *VEGF*, *MMPs*, and *MYLK*—collectively promoting invasion and vascular leakage (38). Systemically, however, elevated *IL-18* levels may signify an ongoing *Th1/NK* cell-mediated anti-tumor immune response, reflecting the host’s attempt to mount immune surveillance against metastatic clones. This “inflammation-mechanics coupling” framework reconciles the apparent contradiction: high *IL-18/MYLK* expression marks tumors that have elicited a host defense response—immune activation accompanied by structural stabilization—which, despite causing local tissue damage, ultimately constrains metastatic progression and prolongs

survival (39).

Clinical translation: prognostic model and therapeutic opportunities

Building on this mechanistic understanding, our *IL-18/MYLK*-based risk model provides a clinically actionable tool that quantifies the state of the neuro-immune microenvironment (40). The model's excellent performance in independent validation cohorts underscores its robustness for identifying “defense-deficient” patients—those with low *IL-18/MYLK* expression who lack effective immune and structural protection and are at highest risk for rapid progression (41). For these patients, intensified imaging surveillance and early multimodal intervention may be warranted (42).

The identification of *PD98059* as a dual *IL-18/MYLK* inhibitor opens therapeutic avenues specifically targeting the inflammation-mechanics coupling we have identified (43). By simultaneously blocking upstream inflammatory signaling (*IL-18*) and downstream cytoskeletal effector (*MYLK*), *PD98059* may “normalize” the metastatic niche—reducing pathological vascular permeability while preserving systemic immune function (44). This “microenvironment engineering” approach represents a paradigm shift from conventional therapies that focus exclusively on tumor cell killing, instead targeting the neuro-immune ecosystem that enables metastasis (45).

Limitations and future directions

Several limitations warrant consideration. First, our findings are based on transcriptomic data and computational predictions; causal relationships between *IL-18* and *MYLK*, as well as the functional effects of *PD98059*, require experimental validation through *in vitro* and *in vivo* models (46). Second, the spatial transcriptomics sample size ($n=2$) is limited; expansion to larger cohorts with diverse molecular subtypes will be necessary to capture the full heterogeneity of BCBM microenvironments (47). Third, our prognostic model, while robust in available cohorts, requires prospective validation in multi-ethnic populations, particularly Asian patients who may exhibit distinct genetic and immune backgrounds.

Future studies will focus on three directions aligned with our integrated framework: (I) establishing causal links between *IL-18* and *MYLK* via *CRISPR*-based gene editing to confirm the inflammation-mechanics coupling

mechanism; (II) evaluating *PD98059* efficacy in preclinical BCBM models to validate its dual-target activity within the neuro-immune microenvironment; and (III) prospectively validating our prognostic model in multicenter clinical cohorts to translate microenvironmental states into clinical decision-making.

Conclusions

This multi-omics study systematically characterizes the BCBM neuro-immune microenvironment through an integrated pipeline spanning single-cell dissection, spatial mapping, clinical validation, prognostic modeling, and drug screening. Our findings establish macrophages as the central signaling hub orchestrating a pro-metastatic ecosystem, and identify the *IL-18/MYLK* axis as a key “inflammation-mechanics coupling” mechanism driving metastatic colonization while paradoxically serving as a systemic marker of protective host responses. The *IL-18/MYLK*-based risk model provides a robust tool for patient stratification, and the dual-target inhibitor *PD98059* represents a promising candidate for microenvironment-normalizing therapy. These interconnected findings offer a comprehensive framework for understanding BCBM pathogenesis and provide translational foundations for precision risk assessment and targeted intervention.

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Footnote

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aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments.

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