Spatial Transcriptomic Profiling Reveals Neurotransmitter-Driven Vulnerabilities in Stress-Associated TNBC Brain Metastases

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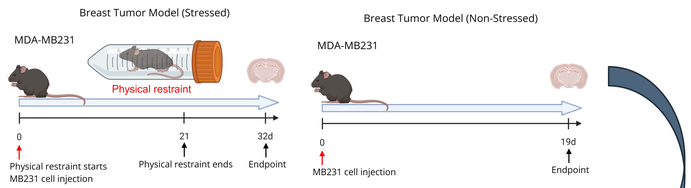
Introduction

- Brain metastasis from triple-negative breast cancer (TNBC) is highly aggressive and disproportionately affects African American patients in Texas.
- Chronic psychological stress—common in over 50% of cancer patients—disrupts neurotransmitter homeostasis and is increasingly linked to cancer progression and metastasis.

Purpose

- Among stress-related neurotransmitters, serotonin (5-HT) is elevated under chronic stress and SSRI use and can worsen brain metastasis.
- This study examines how serotonin signaling drives stress-induced brain metastatic progression in TNBC, using spatial transcriptomics and functional analyses.

Methods



1. Stressed (**A**) and non-stressed (**B**) mouse brain metastasis models were employed.
2. Spatial transcriptomics was performed using the 10X Genomics Visium platform.
3. Tumor and tumor-surrounding clusters were defined based on manual annotation of histological features in H&E-stained sections.

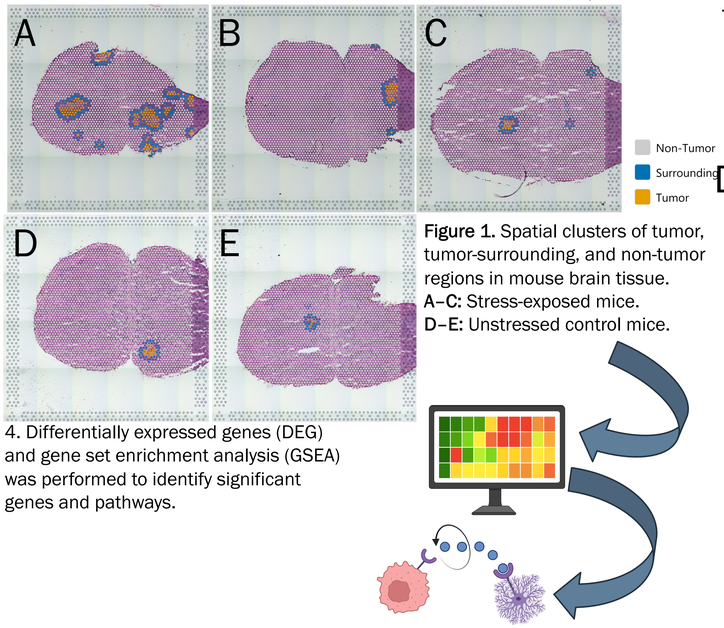


Figure 1. Spatial clusters of tumor, tumor-surrounding, and non-tumor regions in mouse brain tissue.
A–C: Stress-exposed mice.
D–E: Unstressed control mice.

4. Differentially expressed genes (DEG) and gene set enrichment analysis (GSEA) was performed to identify significant genes and pathways.

Results

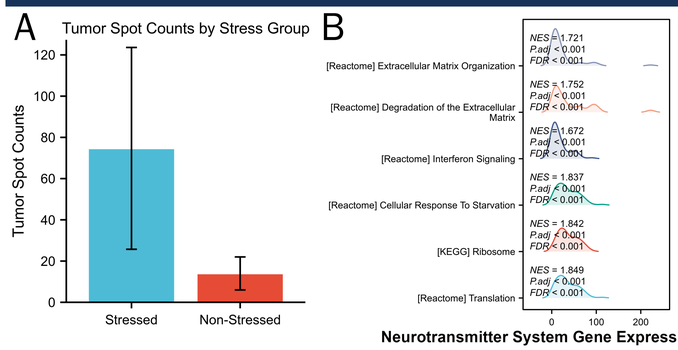


Figure 2. Validation of the chronic stress-induced brain metastasis model.
A: Quantification of tumor-associated spots in chronically stressed versus non-stressed groups.
B: Enrichment of tumor-related pathways in tumor clusters relative to surrounding and non-tumor regions.

Figure 3. Scaled expression of neurotransmitter system genes across tumor and surrounding spatial clusters.

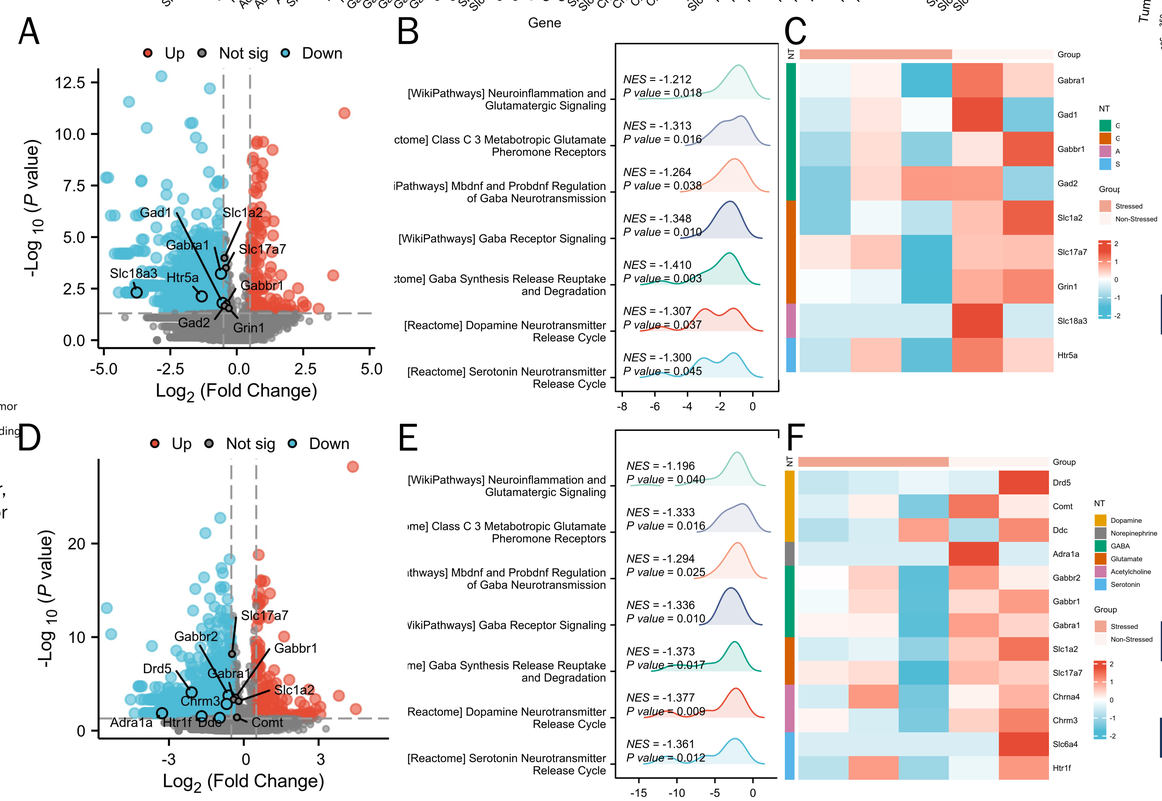


Figure 4. Neurotransmitter DEGs and pathways in chronically stressed versus non-stressed groups.
A, D: DEGs in the tumor (**A**) and surrounding (**D**) clusters. Significant genes are highlighted.
B, E: Enrichment of pathways in chronically stressed versus non-stressed groups in the tumor (**A**) and surrounding (**B**) clusters.
C, F: Normalized expression of DEGs in the tumor (**C**) and surrounding (**F**) clusters.

Results

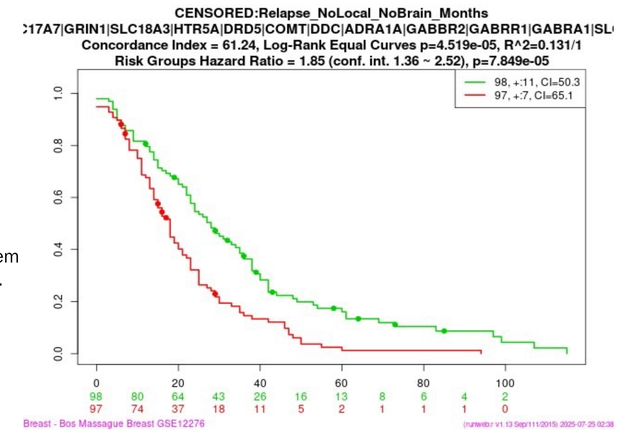


Figure 5. Survival of TNBC brain metastasis patients based on expression of DEGs from Figure 3.

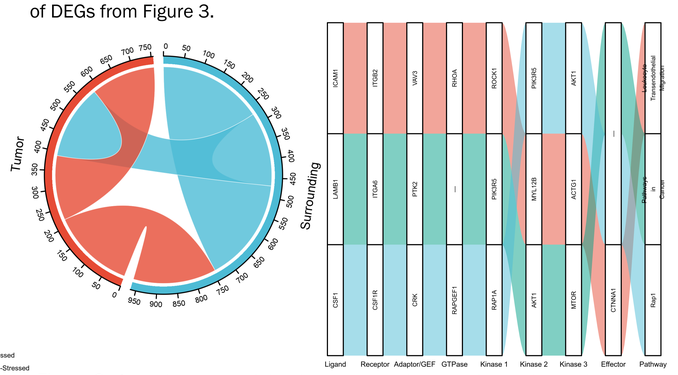


Figure 6. Predicted upregulated ligand–receptor signaling between tumor and surrounding regions:(**A**) overall interactions; (**B**) directional signaling from surrounding to tumor.

Conclusions

- Chronically stressed mice showed increased tumor burden with elevated stress markers and pathways.
- Ongoing studies aim to identify specific stromal and immune cell populations responding to this signaling axis, using spatial transcriptomics-guided multiplex imaging. This work addresses a critical knowledge gap in understanding how stress reshapes the brain metastatic microenvironment.
- Neurotransmitter genes were broadly downregulated under stress, with similar levels in tumor and surrounding tissue.

Acknowledgments

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References

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