Simulated Single-Cell Transcriptomics Reveals Shared Neuroinflammatory Pathways in Age-Related and Radiation-Induced Hippocampal Dysfunction

Danielle Hu[1,6], Katie Ji[2,6], Alex Kuai[3,6], Kailin Xuan[4,6], Haoyi Zhang[5,6]

Great Oak High School, 32555 Deer Hollow Way, Temecula, CA 92592[1]; Lincoln-Sudbury Regional High School, 390 Lincoln Rd, Sudbury, MA 01776[2]; Newton North High School, 457 Walnut St, Newton, MA 02460[3]; The Bishop's School, 7607 La Jolla Blvd, La Jolla, CA 92037[4]; Edgemont Jr./Sr. High School, 200 White Oak Lane, Scarsdale, NY 10583[5]; Boston University, Boston, MA 02215[6]

Introduction

Research Question

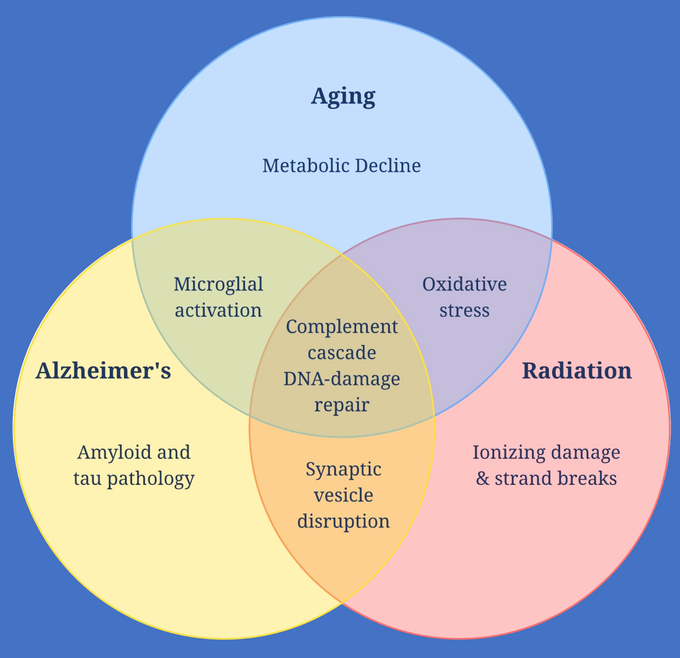
Do non-irradiated, age-related changes in the hippocampus engage the same neuroinflammatory pathways that are activated by cranial radiation therapy?

Hypothesis

Inferred inflammation levels in aging hippocampal tissue will correlate with upregulation of key radiation-response pathways—namely DNA-damage repair (NER, BER, mismatch repair), NF-κB signaling, complement cascade, and synaptic vesicle cycling—mirroring the molecular signature of RT-induced neurotoxicity.

Background

- **Hippocampal Neuroinflammation:** Shared hallmark of normal aging, Alzheimer's disease (AD), and cranial radiation therapy (RT)–induced neurotoxicity
- **RT Molecular Signature:** Microglial activation, cytokine release (IL-1β, TNF-α), complement cascade, DNA-damage repair (BER, NER), NF-κB activation, synaptic vesicle disruption
- **AD Overlap:** Similar upregulation of DNA-repair genes, inflammatory cascades, and neurotransmitter signaling deficits in postmortem hippocampus
- **Current Gap:** Lack of a unified framework comparing RT- and age/AD-driven inflammatory pathways at single-cell resolution
- **Project Aims:**
  - Simulate single-cell–like expression (Splatter) from GSE36980 AD vs. control bulk data, mapped to a DE-ranked gene list
  - Perform subgroup GSEA (top/bottom 10% "inflammation" cells) to identify pathway activation in Control vs. Dementia
  - Test whether non-irradiated aging brains co-opt the same RT-associated neuroinflammatory mechanisms
- **Long-Term Goal:** Highlight shared targets—originally developed for RT neuroprotection—to mitigate age-related hippocampal dysfunction and cognitive decline



Dataset and Tools

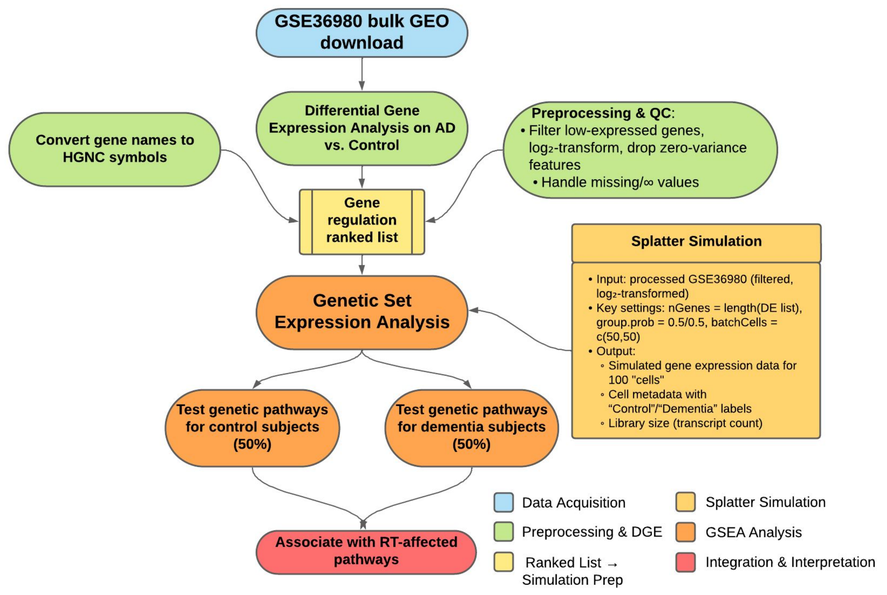
Datasets:

- GSE36980 (GEO): Bulk hippocampal RNA-seq, Alzheimer's disease vs. control postmortem tissue from 80 subjects
- Splatter-simulated scRNA-seq:
  - 10,000 genes × 100 "cells"
  - Two groups (Control vs. Dementia), per-cell library size
- Pathway database:
  - KEGG_2021_Human gene sets (via Enrichr)

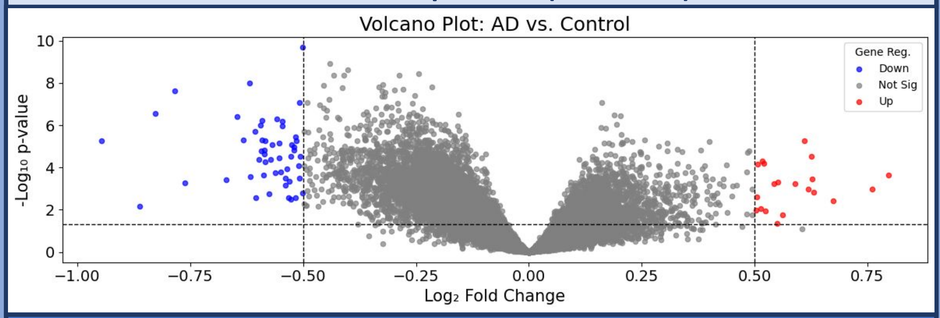
Computational Tools:

Language	Packages & Tools	Purpose
R (v4.x)	GEOquery	Download and preprocess GEO data
	scater	QC, filtering, $\log_2$-transform
	SingleCellExperiment	Store & manipulate SCE objects
	Splatter	Estimate parameters & simulate counts
Python (v3.13	pandas, numpy	Data wrangling & normalization
	scipy.stats (ttest_ind)	Differential statistics
	gseapy	Preranked GSEA on KEGG pathways
	matplotlib	Plotting results

Methodology Overview

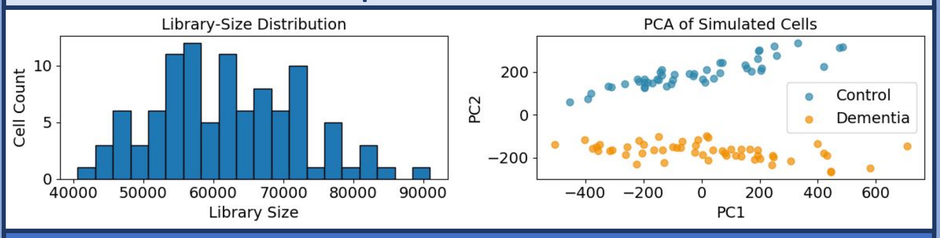


Differential Expression (GSE36980)



- Filter: avg count > 5, var > 0
- Test: limma/DESeq2 → rank by $\log_2$FC & adjusted p-value
- Output: 18 929 DE-ranked genes

Splatter Simulation

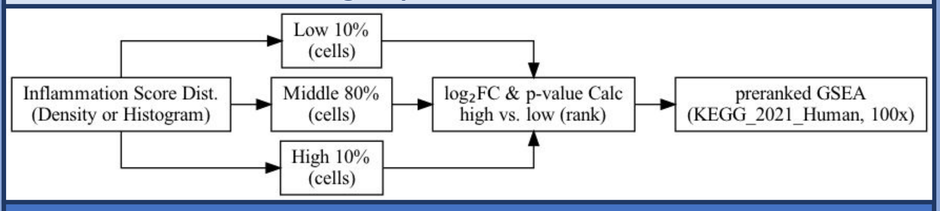


- **Simulation Setup:**
  - 18 929 DE-ranked genes × 100 cells (50 Control, 50 Dementia)
  - group.prob=0.5/0.5, batchCells=(50,50), log-Normal library sizes
- **Outputs:**
  - Counts matrix (genes × cells)
  - Cell metadata with "Control"/"Dementia" labels and library-size factors
- **QC Visualizations:**
  - Library-Size Histogram: confirms realistic log-normal variability in total counts per cell
  - PCA Scatterplot: shows clear separation of Control vs. Dementia cells in PC1–PC2 space, validating simulated group differences

Data Preparation

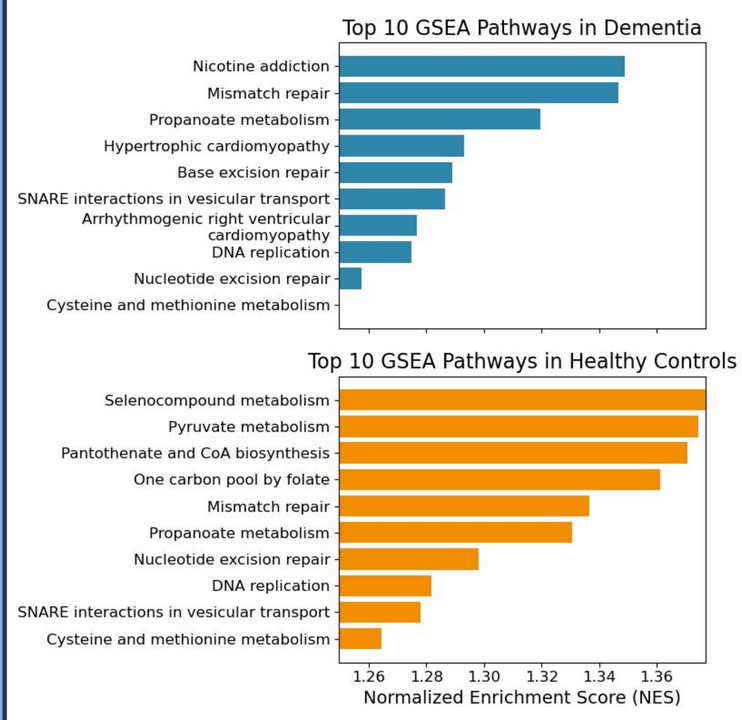
- **Relabel features** → DE-ranked genes
- **Map Group1/Group2** → Control/Dementia
- **Compute inflammation score:**
  - score = library_size / max(…)

Subgroup Selection & GSEA

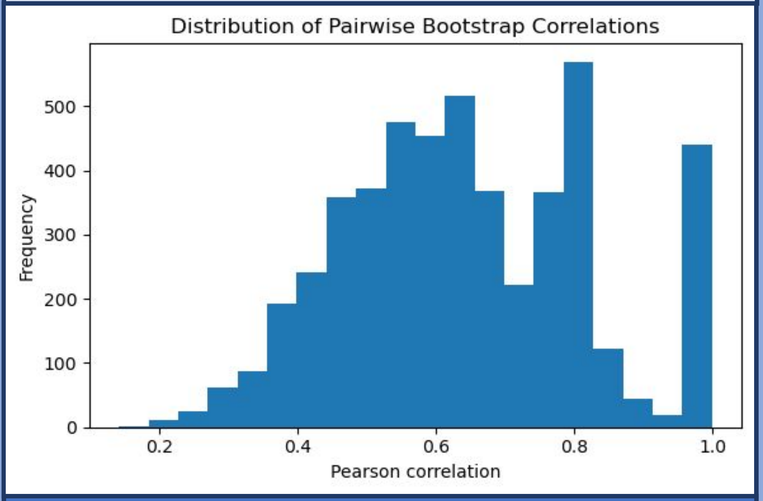


- Stratify cells by inflammation score within each diagnosis (top 10% vs. bottom 10%)
- **Compute gene rankings:**
  - $\log_2$ fold-change = mean(high) – mean(low)
  - two-sample t-test p-values
- Generate preranked lists sorted by logFC for Control and Dementia
- **Run GSEA** (gseapy.prerank on KEGG_2021_Human, 100 permutations)
- **Output:** top enriched pathways (NES, FDR) for each subgroup

Results



Validation



- **Bootstraps & comparisons:** 100 subsamples → 4 950 unique pairwise correlations
- **Mean ρ (average similarity):** 0.644
- **SD ρ (variability):** 0.178
- **Majority of ρ (0.4–0.8):** indicates moderate reproducibility
- **Low ρ (<0.4):** reveals some subsamples with divergent NES profiles
- **Spike at 1.0:** reflects self-comparisons or identical-output artifacts
- **Next steps:** increase GSEA permutations & refine sampling to target ρ ≥ 0.8

Algorithm Validation

- Performed 100 bootstrap subsamples (80 % of Dementia cells, with replacement)
- Reran GSEA on each subsample to obtain NES profiles
- Calculated pairwise Pearson correlations across all replicate NES vectors
- Observed mean ρ = 0.64 (SD = 0.18), indicating consistent enrichment patterns
- Demonstrates that our pipeline reliably recovers pathway signals despite sampling variability

Discussion

Conclusions

- Enrichment of **Mismatch Repair**, **Nucleotide Excision Repair**, and **DNA Replication**: all core ionizing-radiation response pathways
- **Base Excision Repair**, which specifically processes RT-induced single-strand breaks and small base lesions, is uniquely upregulated in Dementia, suggesting enhanced handling of radiation-like genomic results in neurodegeneration
- The shared and differential engagement of these pathways implies that neuroinflammatory stress in Dementia may capture aspects of RT-induced DNA damage response

Limitations

- GSEA reflects transcriptomic signatures, not direct measures of DNA lesion burden or repair enzyme activity
- Moderate bootstrap stability (mean pairwise ρ=0.64) entails careful interpretation

Future Research

- Validate RT-like repair activation with biochemical assays (e.g. γH2AX foci, BER enzyme activity) in brain tissue
- Broaden gene-set analyses to include RT-specific damage sensors (e.g. ATM/ATR signaling)
- Correlate DNA-repair signatures with neuroinflammatory markers to probe mechanistic overlap with RT neurotoxicity

Acknowledgements

We would like to thank Karla Montejo, Shankar Ramachandran, Piergiulio Bressan, and all of the teaching fellows for their mind-opening instruction in computational neurobiology. We are also incredibly grateful for the support from our families and to Boston University for allowing us to experience a fantastic opportunity at RISE.

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