Spatiotemporal single-cell profiling between AD and TBI reveals dysregulated pathways as potential therapeutic targets

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Abstract

Traumatic brain injury (TBI) is a known risk factor for Alzheimer Disease (AD). Both TBI and AD have been found to display similar neurological symptoms and have increasing incidences of disability, morbidity, mortality, and economic burden. Despite these connections, the precise molecular relationship between the two remains relatively underexplored. Single-cell RNA-sequencing (scRNA-seq) has allowed for a progressive understanding of the cellular and genetic landscapes of TBI and AD. This study repurposes single-cell data from the frontal cortex and hippocampus collected at the acute (24-h) and subacute (7-day) phases from mice that underwent fluid percussion injury (FPI) (Arneson et al., 2022). We compared this data to single-cell human data from the parietal cortex of postmortem AD brains (Brase et al., 2021). This includes carriers of pathogenic variants (*APP, PSEN1* and *PSEN2*), also referred to as autosomal dominant AD, and individuals with sporadic AD. We examined the molecular overlap of differentially expressed genes in FPI TBI brains with those differentially expressed in AD brains to identify cell-type-specific biological pathway comparisons across different timescales. Astrocytes and activated microglia, being the most transcriptionally altered cells in the TBI data, were the primary targets of investigation. Statistical analyses revealed substantial molecular resemblance of DEGs co-expressed in both TBI and AD brains in astrocytes, as well as in activated microglia. Our results show specific pathways dysregulated in astrocytic and glial cells in the acute and subacute phases of TBI mouse models that resemble those dysregulated in human postmortem AD brains. By understanding the molecular similarities and differences at single-cell resolution between TBI and AD in a spatiotemporal manner, we aim to uncover specific pathways dysregulated in TBI that contribute to AD etiology.

Introduction

Neurodegenerative diseases represent a significant and escalating global health challenge, posing major implications for affected individuals, their families, and healthcare systems (Martin Prince et al., 2015). It is estimated that 13.9 million Americans will be living with Alzheimer disease (AD) and related dementias by 2060 (Matthews et al., 2019). With an aging population, the prevalence of these disorders has surged, placing an unprecedented burden on societies worldwide. The increasing prevalence of neurodegenerative diseases underscores the urgent need for comprehensive research to unravel their complex mechanisms and identify potential therapeutic targets. Addressing the challenges posed by these disorders requires an understanding of the biological, genetic, and environmental factors contributing to their onset and progression.

Alzheimer Disease

Alzheimer disease stands as the most prevalent among dementia-related disorders. It is characterized by progressive memory loss, a decline in cognition, and changes in behavior, which can ultimately lead to a loss of independence and fatality (Brase et al., 2021 and Mielke et al., 2022). AD poses substantial health and economic burdens. Age is the primary risk factor for Alzheimer disease, but studies have identified genetic markers associated with increased risk of AD (Brase et al., 2021). This irreversible neurodegenerative condition manifests in two primary forms: sporadic Alzheimer disease (sAD) and autosomal dominant Alzheimer disease (ADAD). Within sporadic AD, a notable hallmark resides in genetic pathogenic variants involving *apolipoprotein E* (*APOE*) ε4, serving as a significant risk factor for disease susceptibility and progression of AD pathogenesis. ADAD is predominantly characterized by genetic carriers

harboring pathogenic variants within amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) (Brase et al., 2021). The presence and aggregation of amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFTs) are thought to contribute to a cascade of cellular changes, potentially involving increased neuroinflammatory responses, altered synaptic function, disrupted myelination processes, and apoptotic activity in the brain, though this remains a topic of ongoing debate. (Serrano-Pozo et al., 2011). The effects of these processes further exacerbate irreversible neuronal dysfunction, compromising the signal transmission and neuronal connectivity in afflicted brain regions.

Traumatic Brain Injury

Traumatic brain injury (TBI) has been found to be a significantly contributing risk factor to developing Alzheimer disease and dementia-related diseases (Dams-O'Connor et al., 2016 and Mielke et al., 2022). More than 223,050 Americans are hospitalized due to TBI each year (Peterson and Thomas, 2018). This number is further increased by the widespread occurrence of TBIs attributable to an epidemic of motor vehicle accidents, incidents affecting the elderly population, athletes engaged in high-impact sports, and individuals serving in the armed forces, collectively posing a substantial threat to individuals across diverse demographics on a global scale (CDC, 2021). The progression of TBI can be divided into two distinct phases: the subacute phase, spanning from the immediate impact to 24 hours post-injury, and the acute phase, the period beyond the initial 24-hour period. Distinctions in pathological hallmarks have emerged between these distinct temporal phases, emphasizing the evolving landscape of TBI-induced

neurological effects. The primary phase of TBI primarily results from the immediate impact, encompassed by axonal shearing, cerebral edema, and the perturbation of cerebral blood vessels within the architecture of the brain (John Hopkins Medicine, n.d.). The secondary phase is marked by an exacerbation of neuroinflammation, excitotoxicity, the formation of glial scars, disruptions in mitochondrial, lysosomal and autophagic pathways, and apoptotic activity (John Hopkins Medicine, n.d.). These processes ultimately contribute to the similar neurological symptoms observed in affected individuals with AD, such as memory loss, difficulty concentrating, behavioral changes, and disorientation (Brase et al., 2021, Dams-O'Connor et al., 2016, Mielke et al., 2022, and Sharm et al., 2021).

Neurobiological mechanisms: astrocytes and activated microglia in AD and TBI

Studies have shown that both AD and TBI both affect the frontal cortex and hippocampus (Arneson et al., 2022). The frontal cortex is associated with processes such as problem solving, memory, and judgment, while the hippocampus is involved in processes such as learning and memory. Cognitive functions associated with these regions, such as memory, problem-solving, and judgment, are compromised in both conditions.

Astrocytes - "star-shaped" glial cells - are abundant non-neuronal cells that provide support to neurons within the CNS. They contribute significantly to nervous system health by regulating the chemical environment around neurons, maintaining ion balance, providing metabolic support, and participating in the repair and scarring processes following injury to the brain (Haydon and Nedergaard, 2015). Astrocytes also play a vital role in modulating synaptic activity and are integral to various brain functions, including synapse formation, neurotransmitter

recycling, and regulating blood flow in response to neuronal activity (Haydon and Nedergaard, 2015).

Microglia, on the other hand, are the resident macrophages of the CNS and serve as the principal mediators of the brain's immune defense. These specialized cells continuously monitor the brain microenvironment and swiftly respond to any disturbances or insults, acting as the first line of defense against pathogens, injury, or aberrant cellular components. When activated, microglia undergo morphological and functional changes, transitioning into an activated state characterized by increased phagocytic activity, elevated production of inflammatory mediators, such as cytokines, chemokines, and reactive oxidative species, and the ability to migrate to the site of injury or pathology (Colonna and Butovsky, 2017 and Edison et al., 2012). This activation process is part of the immune response in the brain, aiming to remove damaged cells, clear debris, and initiate repair mechanisms.

Astrocyte and microglia change subcluster state due to TBI

An scRNAseq study performed on a TBI model of a mouse identified cell types that are altered due to a traumatic brain injury. Astrocytes and activated microglia appeared to be the top two most transcriptionally altered cells at both the acute (24-hrs) and the subacute (7 days) phases (Arneson et al., 2022). Depending on the nature of the stimulus, different cells can take on a variety of altered cell-specific states from their normal state (Gottlieb et al., 2022). This includes resting, activated, reduced-activation, pro-inflammatory, and stressed states. Each altered cell state can vary per spatiotemporal region and corresponds to a different morphology and function.

In the context of traumatic brain injury (TBI), astrocytes and microglia respond rapidly to injury, becoming highly transcriptionally altered cells. This heightened sensitivity allows these cell types to undergo changes in their gene expression patterns in response to the injury, contributing significantly to the cellular and molecular alterations observed in the injured brain. Specifically, astrocytes and activated microglia play pivotal roles in creating neuroinflammatory responses, promoting tissue repair, and influencing the overall pathological progression following TBI (Schimmel, Acosta, and Lozano, 2017).

Pathway similarities and differences between AD and TBI

Within the architecture of each AD and TBI, studies have shown a dysregulation in molecular and cellular pathways that alter normal function. Both AD and TBI have been found to be associated with chronic neuroinflammation and immune system dysregulation (Schimmel, Acosta, and Lozano, 2017). In AD, neuroinflammation plays a crucial role in disease progression, with activated microglia and astrocytes contributing to neuronal damage. Neuroinflammation has been identified in brain regions exhibiting declined cognitive functions (Lyra e Silva et al., 2021). This inflammatory response, characterized by the activation of microglia and astrocytes, contributes significantly to the pathogenesis of both AD and TBI. Chronic neuroinflammation leads to the release of pro-inflammatory cytokines, reactive oxygen species (ROS), and other neurotoxic molecules (Schimmel, Acosta, and Lozano, 2017). Chronic neuroinflammation can exacerbate neuronal damage and impair cognitive function in affected individuals.

Similarly, TBI triggers a neuroinflammatory response involving astrocytic and microglial activation, release of pro-inflammatory cytokines, and immune cell infiltration, impacting

neuronal survival and repair processes (Haydon and Nedergaard, 2015). Within TBI, exocytotic dysregulation has been an issue that causes increased Ca^{2+} levels within the cell. This causes issues such as cellular degeneration and phagocytic and apoptosis activation. The mammalian target of rapamycin (mTOR) pathway has been linked to increased neuronal degeneration when activated for too long (Arneson et al., 2022). The mitogen-activated protein kinase (MAPK) and nuclear factor-kappa beta (NF-κB) pathways have been linked to increased inflammatory response in response to injury, but if activated for too long, lead to neuronal dysregulation and cell death (Gee et al., 2020). The NF-κB pathway, specifically, has been found to activate microglia as a response (Frakes et al., 2014). Increased activation of microglia can cause a prolonged inflammatory response which can lead to neurodegeneration, as seen in AD. The Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway has been found to be necessary for neuronal cell maintenance. When dysregulated, this pathway can lead to neuroinflammation and further progression of neurodegenerative diseases, as seen in AD (Rusek et al., 2023).

Furthermore, β-amyloidosis - the accumulation of Aβ peptides that lead to the formation of amyloid plaques within the brain - is a hallmark feature of AD pathology. The aggregation of Aβ peptides has long been hypothesized as the primary driver of AD progression. Post-mortem examinations of AD patients have revealed the abundance of Aβ plaques in affected brain regions (Lyra e Silva et al., 2021 and Pontecorvo et al., 2017). Neurofibrillary tangles (NFTs) have been found in post-mortem brains that were diagnosed with AD (Lyra e Silva et al., 2021, Wu et al., 2020). Interestingly, immunohistochemical studies have also demonstrated the presence of Aβ plaques and neurofibrillary tangles (NFTs) in individuals following traumatic brain injury (Wu et al.,

2020), suggesting a potential overlap in the pathological mechanisms underlying AD and TBI. However, despite these findings, the causal relationship between Aβ peptides and the development of AD or TBI remains elusive.

Identifying dysregulated pathways between AD and TBI

Identification of dysregulated pathways in TBI may shine light on mechanisms contributing to AD etiology and dementia-related diseases post-TBI, paving the way for targeted therapeutic interventions. Single-cell RNA sequencing (scRNAseq) has allowed for a progressive understanding of the cellular and genetic landscapes of neurodegenerative conditions such as AD and TBI. The utility of scRNAseq has been instrumental in unraveling the heterogeneity within different cell types and identifying transcriptional alterations occurring at a single-cell level (Tanay and Regev, 2017). By capturing the transcriptomic profiles of individual cells, scRNAseq has unveiled relatively unexplored cellular diversity and dynamic changes in gene expression signatures across various spatiotemporal stages and regions of the brain affected (Soreq et al., 2023). The application of scRNAseq in AD research has shed light on the intricate interplay between diverse cell types, such as neurons, astrocytes, microglia, oligodendrocytes, and endothelial cells (Soreq et al., 2023). Utilizing scRNAseq data allows for the identification of differentially expressed genes (DEGs), allowing for the identification of genes that exhibit statistically significant changes between diseases. By utilizing scRNAseq, researchers have also identified potential therapeutic targets and candidate biomarkers for many diseases worldwide.

We hypothesize that there is cellular crosstalk at the pathway level between AD and disease and TBI as evidenced by shared gene expression in astrocytes and activated microglia.

To further investigate previous findings on AD and TBI, we integrated scRNA-seq data from the dorsolateral prefrontal cortex of human donors from the Dominantly Inherited Alzheimer Network (DIAN) and Charles F. And Joanne Knight Alzheimer Disease Research Center (Knight ADRC) biobanks that were previously diagnosed with AD, and the frontal cortex and hippocampus of fluid percussion injury (FPI) mouse model (see Methods). Using mouse TBI models allowed for the study of early neurodegenerative changes that may lead to AD, helping to identify potential biomarkers for earlier detection than through a longitudinal study in humans. Additionally, the mouse TBI model offers a more ethical approach to investigating disease progression and testing interventions. Different brain regions were compared as well to identify overlapping and different biological markers in both mice and humans. Through this study, we identified upregulated differentially expressed genes (DEGs) between the two cohorts in specific cell states. Statistical analyses were performed to identify significant spatiotemporal regions. Finally, pathway analyses were performed to identify dysregulated pathways in TBI that contribute to AD etiology. This is the first study to identify a cellular-crosstalk association between AD and TBI at the single-cell level between mice and human brains.

Methodology

Data sets

The scRNA-seq data sets utilized in this study were obtained from the works of Arneson et al. (2022) and Brase et al. (2021). The dataset from Arneson et al. (2022) consists of single-cell RNA sequencing (scRNAseq) data derived from a traumatic brain injury (TBI) mouse model

subjected to fluid percussion injury. Samples were taken from the frontal cortex and hippocampus at two distinct post-injury timepoints: 24 hours, representing the acute phase, and 7 days, representing the subacute phase. In contrast, the dataset from Brase et al. (2021) includes scRNAseq data from post-mortem human brain samples of individuals with sporadic (sAD) or autosomal dominant Alzheimer disease (ADAD), focusing on the dorsolateral prefrontal cortex. These human samples were obtained from the Dominantly Inherited Alzheimer Network (DIAN) and the Knight ADRC biobanks, while the mouse samples came from C57BL/6 J mice that experienced fluid percussion injury to model TBI.

Cell expression state clustering

The clustering of cell expression states followed the methodology outlined by Brase et al. (2021). Single Nucleus Alzheimer disease RNA Explorer (SNARE) webtool by the Harari Lab was utilized to identify subcluster of co-expression between data sets.

Statistical analyses

R Studio software was used to filter Arneson et al. (2022) and Brase et al. (2021) data sets and run statistical tests, including Fisher exact tests, to assess the statistical significance of gene co-expression within the data sets at both acute (24-h) and subacute (7-day) phases. Statistical significance was determined by a p-value lower than 0.05 at a 95% confidence interval.

Pathway analysis

The Enrichr web-based tool by Ma'ayan Laboratory (Chen et al., Kuleshov et al., and Xie Z et al., 2021) was utilized to identify pathways that were dysregulated in both TBI and AD (sAD and

ADAD). KEGG, Reactome 2022, MSigDB Hallmark 2020, and WikiPathway gene sets were used in determining pathway enrichments.

Code availability

R Studio scripts produced to analyze the data sets in the current study are available upon request from the corresponding author.

Results

Differential co-expression patterns in astrocytes and activated microglia

Single Nucleus Alzheimer Disease RNA Explorer (SNARE) mapping allows for gene expression in different cell type subclusters to be identified (Harari Lab, n.d.). The subclusters are representative of the various states that the cell can take on during injury. SNARE mapping of differentially expressed genes (DEGs) in AD in the frontal cortex and TBI in the frontal cortex and hippocampus was performed to identify co-expression subcluster regions. Identifying co-expression allowed us to determine in which cell state astrocytic genes in both AD and TBI were expressed. SNARE mapping of astrocytes divided astrocytes into subcluster groups consisting of Astro. 0-3 (Astro. 0 = ‘resting’, Astro. 1 = ‘activated’, Astro 2 = ‘reduced activation’, Astro 3 = ‘pro-inflammatory’) (Figure 1a). SNARE mapping of frontal cortex astrocytes revealed that Astro. 0 (‘resting’ astrocytes) was the most transcriptionally shared subcluster between acute TBI and AD DEGs (Figure 1b). SNARE mapping of hippocampal astrocytes revealed that Astro. 0 was also the most transcriptionally shared subcluster between TBI and AD DEGs (Figure 1c).

Activated microglia data are not shown as activated microglia are already in their differentiated state and therefore were not subjected to SNARE mapping.

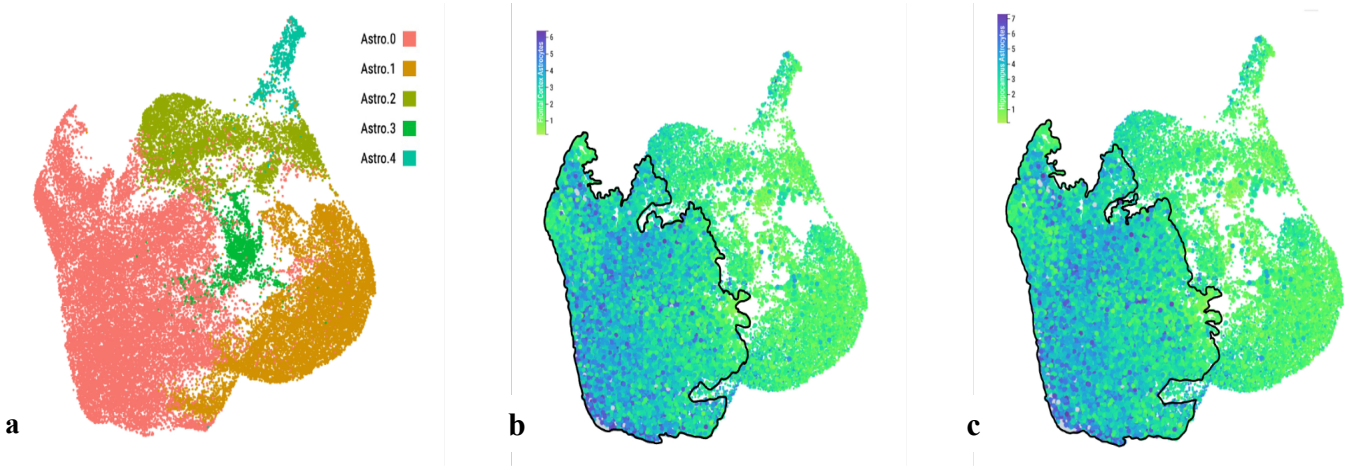


Figure 1: SNARE UMAPs allows for astrocyte gene co-expression subclustering identification
(a) Single Nucleus Alzheimer disease RNA Explorer (SNARE) mapping of Alzheimer genes expressed in different astrocytic subclusters. Astrocyte subclusters divided into: Astro. 0 = 'resting', Astro. 1 = 'activated', Astro 2 = 'reduced activation', Astro 3 = 'pro-inflammatory'. SNARE mapping of AD and TBI genes co-expressed in (b) the frontal cortex and (c) the hippocampus. Colored scale (1: light green to 6: dark blue) represents increased transcriptional sensitivity.

Astrocytes are transcriptionally altered cells in AD and TBI at the acute phase

By identifying shared transcriptional subclusters, the SNARE analysis suggested potential similarities in state subclusters in both the frontal cortex and hippocampus of AD and TBI. To explore potential statistical relationships between astrocytic DEGs in AD and TBI, correlation tests were performed considering a combination of factors, such as whether the TBI injury occurred at the acute or subacute phase, whether the patient was diagnosed with sAD or ADAD, and whether the cells were isolated in the frontal cortex or hippocampus. The original datasets were pooled and then separated to account for these factors. A Fisher exact test was used

to assess the statistical significance of spatiotemporal correlations, with a significance threshold set at a p-value of 0.05 at a 95% confidence interval. Only spatiotemporal points were considered for further analyses.

In astrocytes, the subacute (7-day) phase was not statistically similar in AD versus TBI in both the frontal cortex and hippocampus (data not shown). There appeared to be a positive linear relationship ($r = 0.60$, p-value = 4.34E-02) between the effect sizes of log fold change (logFC) of transcriptional alteration in comparison to sham in astrocytes of sAD versus TBI at the acute phase in the frontal cortex (Figure 2a). In ADAD versus TBI, there also appeared to be a positive linear relationship ($r = 0.47$, p-value = 6.32E-05) in the frontal cortex of associated genes in all astrocytes within the AD and TBI datasets (Figure 2b).

Fisher exact tests revealed that astrocytic DEGs expressed between AD and TBI were statistically similar to one another in these conditions. Correlation tests were performed on spatiotemporal points in the hippocampal astrocytes. In sAD versus TBI, there appeared to be a strong positive linear relationship ($r = 0.71$, p-value = 7.32E-03) between the effect sizes at the acute phase in the hippocampus (Figure 3a). In ADAD versus TBI, there also appeared to be a positive linear relationship ($r = 0.41$, p-value = 1.39E-02) (Figure 3b).

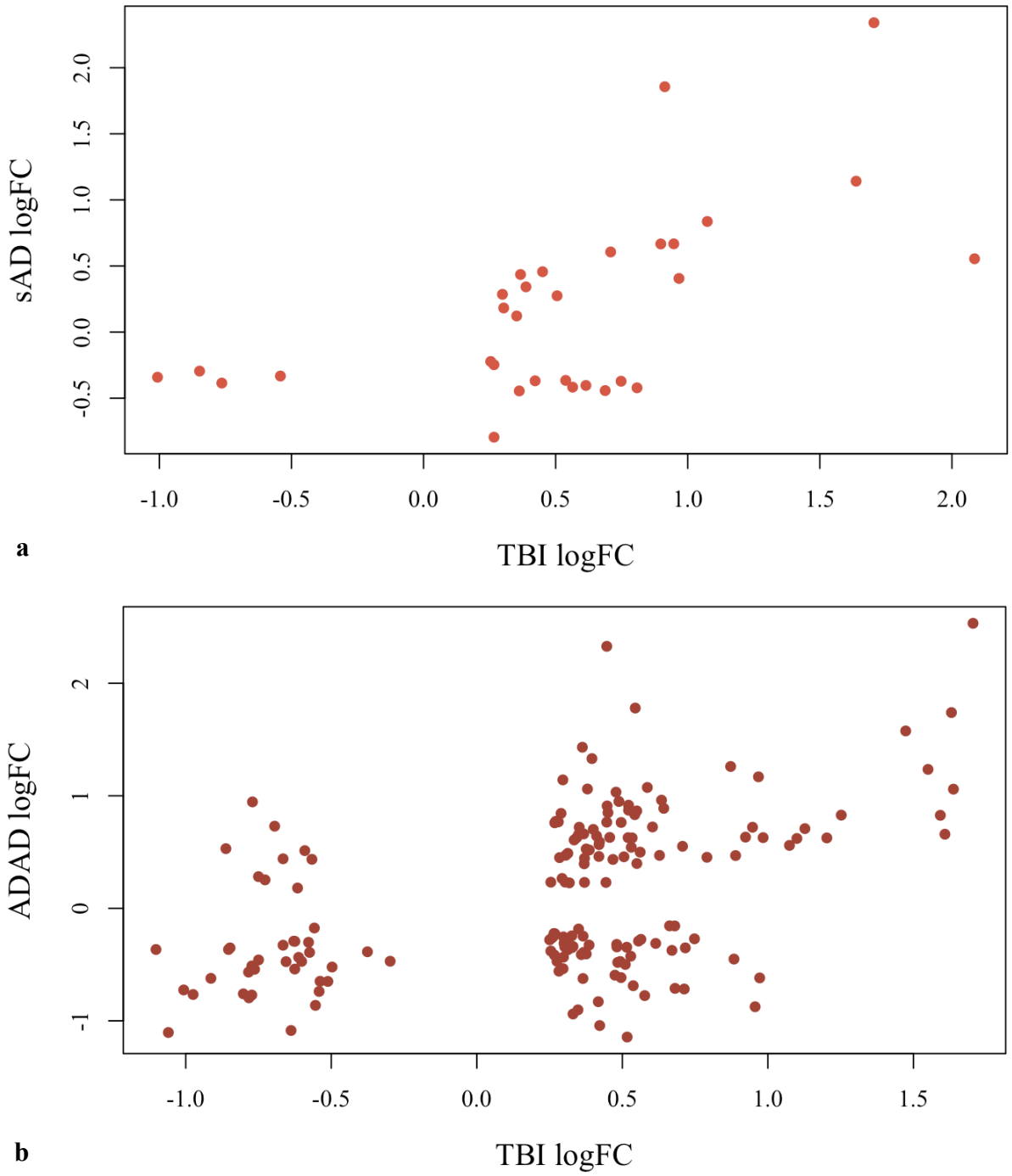
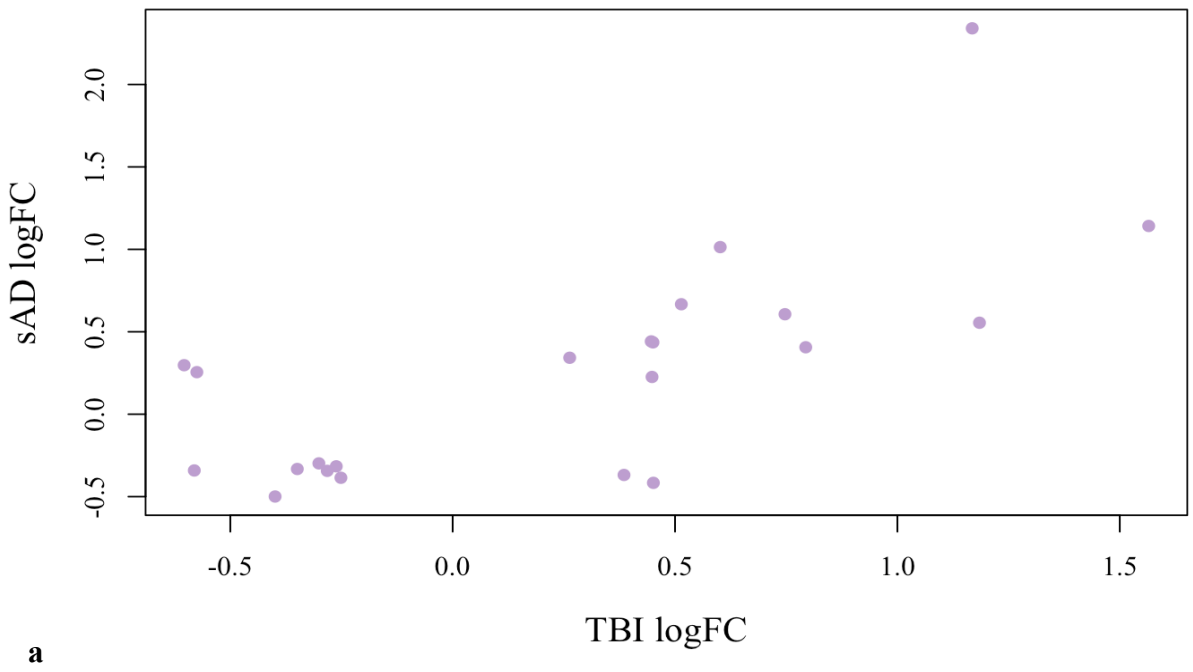
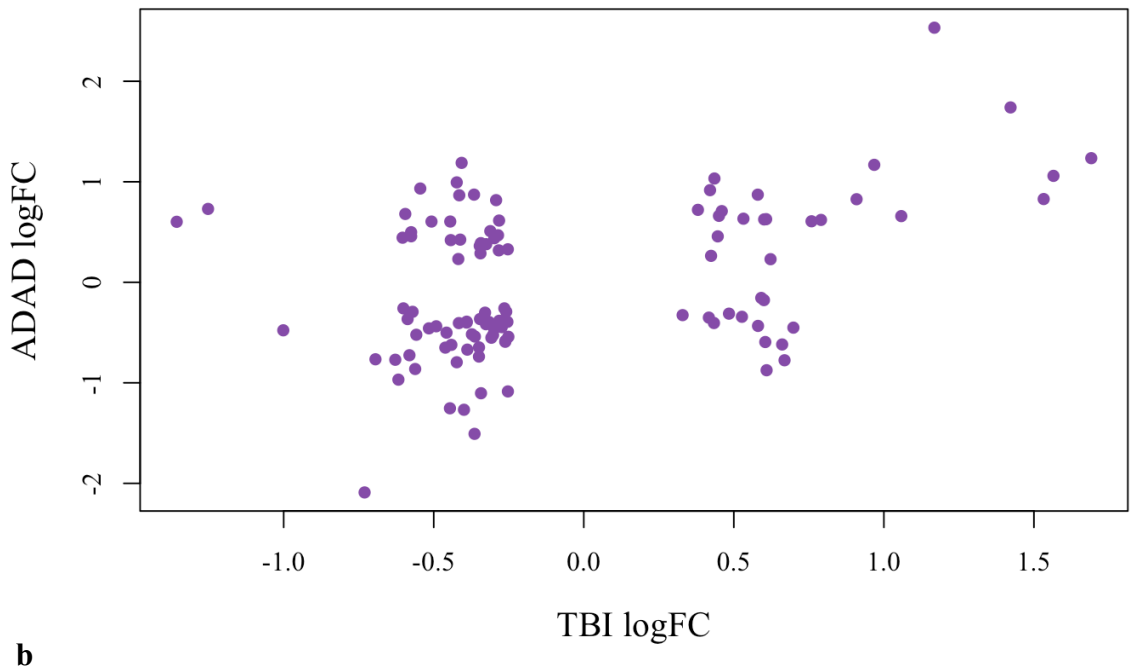


Figure 2: Statistical comparison of astrocytes: TBI 24-hr versus sAD and ADAD (Frontal Cortex)

(**a**) Number of differentially expressed genes co-expressed in astrocytes within the frontal cortex between traumatic brain injury (TBI) model mice brains at the 24-hr (acute) phase versus post-mortem human sporadic Alzheimer disease (sAD) (r = 0.60, p-value = 4.34E-02) and (**b**) ADAD brains (r = 0.47, p-value = 6.32E-05). LogFCs of each co-expressed gene were co-analyzed. A Fisher exact test was performed to assess statistical significance.



a



b

Figure 3: Statistical comparison of astrocytes: TBI 24-hr versus sAD and ADAD (Hippocampus)
(a) Number of differentially expressed genes co-expressed in astrocytes within the hippocampus between traumatic brain injury (TBI) model mice brains at the 24-hr (acute) phase versus deceased human sporadic Alzheimer disease (sAD) (r = 0.71, p-value = 7.32E-03) (**b**) and ADAD brains (r = 0.41, p-value = 1.39E-02). LogFCs of each co-expressed gene were co-analyzed. A Fisher exact test was performed to assess statistical significance.

Activated microglia are transcriptionally altered cells in ADAD and TBI at the acute phase

In addition to astrocytes, our goal was to identify whether activated microglia, the second most transcriptionally altered cell type in TBI, had a causal relationship between AD and TBI. While our analysis primarily focused on identifying similarities in gene expression patterns and dysregulated pathways between AD and TBI, establishing shared gene expression patterns in activated microglia serves as a foundational step toward elucidating potential causal connections. By examining the transcriptional responses of activated microglia in both AD and TBI, we tested to identify whether certain gene expression patterns within these cells may act as contributors to the etiology of AD post-TBI, thus suggesting a statistical link between the two neurodegenerative conditions. This investigation aimed to shed light on the role of activated microglia in bridging the gap between AD and TBI. A Fisher exact test determined that the acute phase did not reach a level of statistical significance in sAD versus TBI in both the frontal cortex and hippocampus (data not shown). In the acute phase of ADAD versus TBI, significance was not reached in the hippocampus (data not shown). The subacute phase also did not reach a level of statistical significance in sAD and ADAD versus TBI in both the frontal cortex and hippocampus (data not shown). In the acute phase, there appeared to be a weak yet linear positive relationship ($r = 0.32$, p-value = 4.39E-03) between the effect sizes of ADAD versus TBI in the frontal cortex (Figure 4).

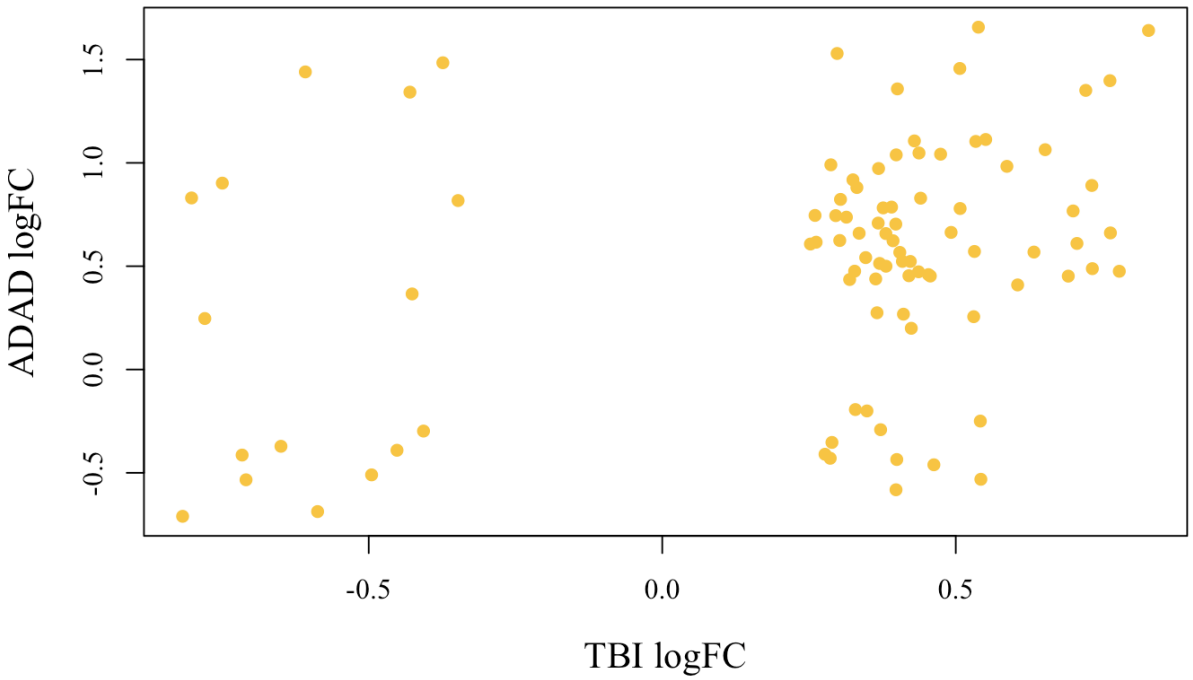


Figure 4: Statistical comparison of activated microglia: TBI 24-hr versus ADAD (Frontal Cortex)
Number of differentially expressed genes co-expressed in activated microglia within the frontal cortex between deceased human sporadic Alzheimer disease (ADAD) brains and traumatic brain injury (TBI) model mice brains at the 24-hr (acute) phase. LogFCs of each co-expressed gene were co-analyzed (r = 0.32, p-value = 4.39E-03). A Fisher exact test was performed to assess statistical significance.

Astrocytes and activated microglia have similar and divergent genetic dysregulations in the frontal cortex and hippocampus of AD and TBI

By identifying which spatiotemporal combinations produced a p-value below 0.05, we were able to identify cellular pathways that were deregulated in astrocytes and activated microglia in both AD (sAD and ADAD) and TBI. By identifying enriched pathways, we can expand on awareness of pathways that may become upregulated or downregulated post-TBI that may lead to possible AD etiology. The webtool, Enrichr, was used to perform pathway analyses at each spatiotemporal point that was determined previously to be statistically significant (p-value < 0.05 at a 95% confidence interval). The enriched pathways from various gene sets that

appeared to follow a similar level of statistical significance are shown, along with their adjusted p-values (q-values) and the overlapping genes that were co-expressed between TBI and AD (Tables 1 and 2).

In sAD versus TBI of the acute phase, the JAK/STAT, apoptosis, cytokine signaling, interleukin-6 family signaling, and signaling by interleukins pathways were enriched in astrocytes in both the frontal cortex and hippocampus (Tables 1 and 3). Many genes overlapped and were expressed in multiple pathways at this spatiotemporal point, including *SOCS3*, *STAT3*, *OSMR*, *CD44*, and *TNFRSF12A* (Tables 1 and 2). In contrast, the reactive oxygen species (ROS) pathway was dysregulated in the hippocampus (Table 2), but not in the frontal cortex at this stage.

Table 1: Pathway analysis of astrocytes in the frontal cortex in sAD versus TBI (acute phase)

PATHWAY	Q-VALUE	OVERLAPPING GENES
JAK-STAT signaling pathway	4.97e-04	[*SOCS3, STAT3, OSMR, GFAP*]
Apoptosis	4.78e-05	[*TNFRSF12A, EMP1, SQSTM1, CD44*]
Cytokine Signaling In Immune System R-HSA-1280215	8.10e-05	[*SOCS3, TNFRSF12A, HNRNPDL, STAT3, OSMR, SQSTM1, CD44*]
Interleukin-6 Family Signaling R-HSA-6783589	8.10e-05	[*SOCS3, STAT3, OSMR*]
Signaling By Interleukins R-HSA-449147	2.07e-05	[*SOCS3, HNRNPDL, STAT3, OSMR, SQSTM1*]

Table 2: Pathway analysis of astrocytes in the hippocampus in sAD versus TBI (acute phase)

PATHWAY	Q-VALUE	OVERLAPPING GENES
JAK-STAT signaling pathway	4.27e-03	[*STAT3, OSMR, GFAP*]
Apoptosis	1.44e-02	[*TNFRSF12A, CD44*]
Cytokine Signaling In Immune System R-HSA-1280215	1.99e-03	[*TNFRSF12A, HNRNPDL, STAT3, OSMR, CD44*]
Interleukin-6 Family Signaling R-HSA-6783589	3.70e-03	[*STAT3, OSMR*]
Signaling By Interleukins R-HSA-449147	3.40e-02	[*HNRNPDL, STAT3, OSMR*]
Reactive Oxygen Species Pathway	6.21e-02	[*PFKP*]

In ADAD versus TBI of the acute phase, the mTORC, apoptosis, Myc Targets V1, and validated targets of C-MYC transcriptional activation pathways were enriched in astrocytes in both the frontal cortex and hippocampus (Tables 3 and 4). Many genes appeared to overlap and be expressed in multiple pathways at this spatiotemporal point, including *NUPR1*, *HSPD1*, *HSP90AB1*, *IFRD1*, *TCP1*, *CCT5*, *CD44*, and *TNFRSF12A* (Tables 3 and 4). In frontal cortex astrocytes, the protein folding and metals and amyloid-beta toxicity in Alzheimer disease pathways were dysregulated as well (Table 3). Notably, the *APP* gene was found to be co-expressed in the metals and amyloid-beta toxicity in Alzheimer disease pathway in the frontal cortex, but not the hippocampus (Table 3). In hippocampal astrocytes, the Tau protein binding pathway was found to be dysregulated (Table 4), but not in the frontal cortex.

Table 3: Pathway analysis of astrocytes in the frontal cortex in ADAD versus TBI (acute phase)

PATHWAY	Q-VALUE	OVERLAPPING GENES
mTORC1 Signaling	1.00e-06	[*SLC7A5, STIP1, HSPA4, IFRD1, UBE2D3, NUPR1, EIF2S2, SQSTM1, HSPD1*]
Apoptosis	3.30e-06	[*DNAJA1, APP, TNFRSF12A, EMP1, HSPB1, SQSTM1, CD44*]
Myc Targets V1	1.00e-06	[*HSP90AB1, PTGES3, RPS5, RPL34, IFRD1, TCP1, EIF2S2, CCT5, HSPD1*]
Validated targets of C-MYC transcriptional activation	1.40e-02	[*HSP90AA1, HSPA4, RPL11, HSPD1*]
Protein Folding	1.63e-03	[*HSPA8, HSP90AA1, ST13*]
Metals and Amyloid beta Toxicity in Alzheimer's Disease	2.52e-02	[*APP, BAG3*]

Table 4: Pathway analysis of astrocytes in the hippocampus in ADAD versus TBI (acute phase)

PATHWAY	Q-VALUE	OVERLAPPING GENES
mTORC1 Signaling	5.93e-03	[*IFRD1, NUPR1, HSPD1*]
Apoptosis	3.63e-02	[*TNFRSF12A, CD44*]
Myc Targets V1	3.90e-05	[*HSP90AB1, IFRD1, TCP1, CCT5, HSPD1*]
Validated targets of C-MYC transcriptional activation	2.54e-02	[*HSP90AA1, HSPD1*]
Tau Protein Binding	6.07e-04	[*HSP90AA1, HSP90AB1, ROCK2*]

A pathway analysis was performed in activated microglia in the frontal cortex in ADAD versus TBI at the acute phase. The Myc Targets V1, mTORC1 signaling, MAK6 signaling, Alzheimer disease, and Parkinson disease pathways appeared to be enriched at this spatiotemporal point of activated microglia (Table 5). Genes that appeared to be repeatedly enriched in various pathways included *HSPA9*, *CCT2*, *HNRNPA3*, *HSP90AB1*, *NOLC1*, *HSPD1*, *CCT6A*, and *EIF4G2* (Table 5).

When comparing pathways enriched in astrocytes and those in activated microglia in the frontal cortex and hippocampus at the acute phase, the Myc Targets V1 and mTORC1 signaling pathways were expressed in the hippocampus of astrocytes and frontal cortex of activated microglia (Tables 3-5). These pathways were not expressed in the frontal cortex of astrocytes. With the Myc Targets V1 pathway, *HSP90AB*, *CCT5*, and *HSPD1* appeared to be repeatedly present. The mTORC1 signaling pathway shared expression of *HSPD1, HSPA4, EIF2S2* (Tables 3-5). The apoptosis pathway was also enriched in astrocytes in both the frontal cortex and hippocampus (Tables 1-4) Expression of *TNFRSF12A*, *EMP1*, *SQSTM1*, and *CD44* was present in both areas (Tables 1-4).

Table 5: Pathway analysis of activated microglia in the frontal cortex in ADAD versus TBI (acute phase)

PATHWAY	Q-VALUE	OVERLAPPING GENES
Myc Targets V1	1.32e-12	[*CCT3, NOP56, CCT2, HNRNPA3, HSP90AB1, HNRNPU, NOLC1, EIF2S2, HSPD1, SERBP1, HNRNPA2B1, CCT5, EIF4G2*]
mTORC1 Signaling	5.69e-04	[*HSPA9*, *CCT6A*, *HSPA4*, *CACYBP*, *EIF2S2*, *HSPD1*]
MAPK6 Signaling	4.56e-10	[*HSPA9, CCT2, HSPA8, HNRNPA3, HSP90AB1, SMARCA5, NOLC1, HSPD1, CCT6A, SERBP1, HNRNPA2B1, CCT8, EIF4G2*]
Alzheimer disease	1.25e-02	[*TUBA1C, PSMC5, PSMB1, LPL, UQCR11, ATP5F1E*]
Parkinson disease	2.11e-03	[*HSPA9, HSPA8, PSMB1, YWHAG*]

Discussion

Currently, there are no cures for either Alzheimer disease or traumatic brain injury. Medications and therapies have been identified in managing symptoms associated with these neurodegenerative disorders (Mayo Clinic, 2024). While these approaches can aid in managing symptoms and improving the quality of life for patients, a definitive cure for AD and TBI remains an area of active research and development in the medical field, while also understanding why TBI can lead to AD in one's lifetime. This analysis of scRNA-seq data from both TBI mouse models and human postmortem AD brains revealed gene expression patterns and shared pathways between the two conditions. This study identified genes that exhibited positive, negative, and non-related expression in both AD (sAD and ADAD) and TBI, highlighting the complex molecular landscape between the two neurodegenerative disorders.

A noteworthy finding is the shared genetic and pathway similarities between TBI and sAD and ADAD. Despite their distinct genetic origins, both forms of AD exhibited similarities in gene expression patterns and dysregulated pathways. This suggests that certain aspects of the neurodegenerative processes, specifically at the molecular level, are shared between sporadic and familial forms of AD with TBI. The identification of these shared elements could have significant implications for understanding common pathological mechanisms underlying AD and other neurodegenerative diseases.

Immunological pathways emerged prominently in the enriched pathways identified in both TBI and AD. The activation of microglia and astrocytes, particularly noted at the acute phase, highlights the involvement of neuroinflammatory responses and cell death processes in both conditions. This aligns with existing literature suggesting a crucial role of neuroinflammation in the progression of both AD and TBI (Kaur U et al., 2015). These findings highlight the importance of these pathways in the acute phase rather than at the subacute phase, emphasizing the early stages as critical points for intervention strategies for dementia-related disorders.

The identification of dysregulated pathways, such as the mTORC1 signaling pathway and Myc Targets V1 pathway, presents insights into the potential mechanisms linking AD and TBI. The dysregulation of these pathways has been implicated in both AD and TBI pathology, suggesting a convergence of molecular pathways underlying neurodegenerative processes. The mTORC1 signaling pathway, known for its role in regulating cellular growth and metabolism, has been implicated in promoting neuronal degeneration when dysregulated (Arneson et al., 2022). In AD, aberrant activation of mTORC1 has been associated with synaptic dysfunction and neuronal loss, contributing

to neurodegenerative disease progression (Arneson et al., 2022). Similarly, in TBI, prolonged activation of mTORC1 signaling has been linked to increased neuronal degeneration and impaired recovery processes (Arneson et al., 2022).

Moreover, the observed dysregulation of the JAK/STAT pathway in both TBI and AD provides further evidence of the connection between neuroinflammatory processes and neurodegeneration. The JAK/STAT pathway plays a crucial role in mediating inflammatory responses and has been implicated in the pathogenesis of various neurodegenerative disorders, including AD and TBI (Rusek et al., 2023). In AD, dysregulated JAK/STAT signaling has been associated with increased microglial activation and pro-inflammatory cytokine production, contributing to neuronal damage and cognitive decline (Rusek et al., 2023). Similarly, in TBI, activation of the JAK/STAT pathway has been linked to exacerbation of neuroinflammation and secondary injury cascades, further perpetuating neuronal dysfunction (Rusek et al., 2023).

Furthermore, the enrichment of pathways related to apoptosis highlights the role of programmed cell death in the pathogenesis of both AD and TBI. Dysregulated apoptosis has been implicated in neuronal loss and synaptic dysfunction, contributing to cognitive impairment and disease progression in AD (Sharma et al., 2021). Similarly, in TBI, dysregulated apoptosis pathways have been linked to increased neuronal cell death and impaired neurodegeneration processes, exacerbating neurological deficits (Arneson et al., 2022). The identification of shared dysregulated pathway signaling highlights the importance of targeting common molecular mechanisms for therapeutic interventions aimed at reducing the risk of AD following a traumatic brain injury.

However, it is crucial to acknowledge the limitations of the study. The data analyzed were derived from mouse models and postmortem human brains. While valuable insights were

gained, the translational relevance to clinical scenarios needs careful consideration. The FPI mouse model was a limitation as the mouse is not subjected to have high alteration of genetic factors as seen in AD mouse models. A possible future study could examine combinations of postmortem human brains with AD with another mouse model, such as the 5xFAD mouse model, that underwent traumatic brain injury. This could possibly allow for the genetic expression of more AD risk genes to be expressed in response to TBI when analyzing scRNAseq data. Additionally, the study focused on specific cell types, primarily astrocytes and activated microglia, potentially missing contributions from other cell populations. Future work should explore a broader spectrum of cell types and validate findings in larger human cohorts to enhance the generalizability of the results. Additionally, more datasets should be utilized to confirm correlations and should be followed using biological functional tests to identify whether these genes and pathways are being altered.

Correlative relationships in genetic expression and pathway expression were present, indicating a potential connection between AD and TBI at the single-cell level. The positive linear relationships identified in astrocytes and activated microglia during the acute phase suggest that certain gene expression changes may act as contributors to the etiology of AD post-TBI. Further investigations into the mechanisms underlying these relationships could unveil novel therapeutic targets for reducing the risk of AD following traumatic brain injuries. This aligns with existing literature highlighting the importance of understanding the molecular similarities and differences between TBI and AD to uncover specific pathways dysregulated in TBI that contribute to AD etiology (Brase et al., 2021 and Arneson et al., 2022). Targeting the co-expressed genes and pathways identified in our study holds promise for reducing the risk of Alzheimer disease following a traumatic brain injury, potentially offering novel therapeutic avenues to mitigate the

neurodegenerative consequences of TBI and prevent or delay the onset of AD and other dementia-related diseases.

Conclusion

In conclusion, this study provides a comprehensive exploration of the molecular relationships between AD and TBI at the single-cell level. The identification of positive, negative, and non-related genes, shared pathways, and causal relationships, particularly in the acute phase, adds valuable insights to our understanding of the potential connections between these neurodegenerative conditions. The emphasis on immunological and apoptosis pathways at the acute phase opens avenues for early targeted therapeutic interventions. However, further research addressing the study's limitations and expanding the scope of cell types analyzed will contribute to further comprehension of the complicated interplay between not only AD and TBI, but also more neurodegenerative disorders. Through identification of co-expressed genes and pathways in AD and TBI, we can discover therapies to reduce the risk of Alzheimer disease and other dementia-related diseases following a traumatic brain injury.

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