Microglia Modulates the Age-effect on Acute TBI Outcomes

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Abstract

Introduction- The CDC estimates nearly 3 million people sustain a traumatic brain injury (TBI) each year. Among them, elders are particularly vulnerable and suffer worse outcomes. Recent work in our laboratory has demonstrated that aged mice had less neuronal loss and preserved white matter connectivity after TBI while young mice demonstrate evidence of neurogenesis. Microglia, the resident innate immune cell of the brain, are complicit in both processes.

Hypothesis- We hypothesized that microglia would adopt age-dependent TBI-associated transcriptional profiles after brain injury.

Methods- We induced brain injury via the controlled cortical impact on two 14-week-old young and 80-week-old aged C57BL/6, respectively. Eight hours later, we harvested the brains and isolated microglia through florescence-activated cell sorting. We utilized single-cell RNA-sequencing to study the molecular identities of microglia isolated from aged brains compared to young brains post-injury.

Results- Differential microglial responses in aged mice compared to young mice were observed with aged microglia adopting a unique phenotype (i.e., enriched inflammatory and immune responses).

Conclusion- This study supports that there are age-dependent transcription responses within the microglia. Thus, we urge that age should be a priori consideration in the future treatment of TBI.

Introduction

Traumatic brain injury (TBI) has long been called the “silent epidemic” because of the low public recognition yet the staggering number of people injured each year, amounting to approximately 3 million in the United States. Among them, elders (≥65 years) are particularly vulnerable and suffer worse outcomes. Moreover, elders offer unique challenges in TBI assessment and clinical management as they are subject to numerous age-related issues such as variable baseline cognitive function, impaired memory, and different comorbid diseases. Although age has been identified as a risk factor for poor TBI outcomes, guidelines for the acute and chronic care of TBI in the geriatric population are lacking. To further address these unique challenges of managing TBI in elders, it is critical to delineate age-linked pathophysiology after TBI.

Recent work in our laboratory has demonstrated that aged mice had less neuronal loss and preserved white matter connectivity after TBI while young mice demonstrate evidence of neurogenesis. To uncover the underlying mechanism contributing to this age-effect, we have focused on microglia, the gatekeepers in the CNS. Since microglial activation plays an indispensable role in neuroinflammation following TBI, we reason that its responses may account for this age-effect. **We hypothesized that microglia would adopt age-dependent TBI-associated transcriptional profiles after brain injury.**

Methods

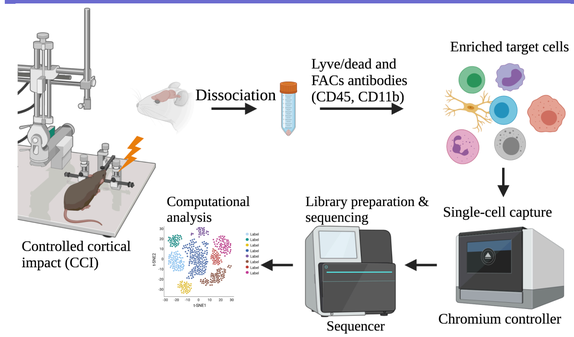


Figure 1. Schematic of 10x Genomic Single-Cell RNA-Seq and CCI TBI model.

- We utilized single-cell RNA-sequencing (scRNA-seq) to study the molecular identities of microglia isolated from injured brains 8 hours post-TBI, i.e., acute TBI.
- Brains were harvested from 14-week-old young and 80-week-old aged C57BL/6 (n=2) after perfusion with heparinized saline.
- Afterward, microglia were identified at CD45^{dim} CD11b^{+}and separated by florescence-activated cell (FAC) sorting. Libraries were prepared using the 10x Genomics Chromium Single Cell 3' Reagent Kits and sequenced on a HiSeq 4000 instrument.
- Raw data were processed using the Cell Ranger pipeline mapped to the mm10 mouse genome. Seurat (version 4.0.1) will be used for clustering and differential gene expression analysis following the standard workflow posted on the Satija Laboratory Website (https://satijalab.org/seurat/index.html).
- Hypergeometric enrichment test was used to examine the enrichment of a cluster in a specific genotype. Ingenuity Pathway Analysis (IPA, QIAGEN) were used for downstream analysis.

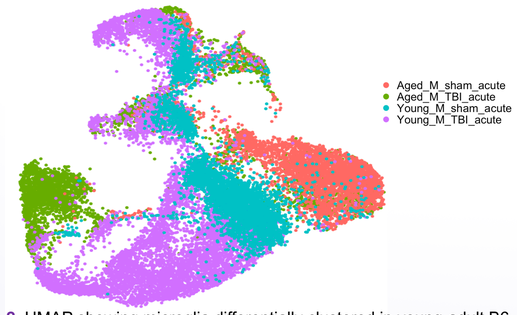
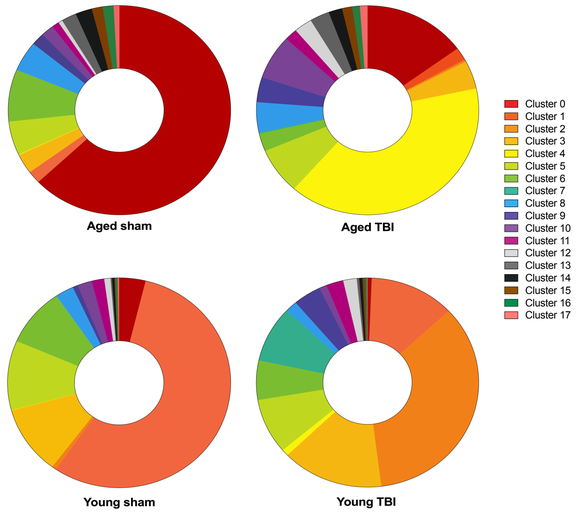


Figure 2. UMAP showing microglia differentially clustered in young-adult B6 mice 8 hours post TBI versus sham and aged B6 mice 8 hours post TBI versus sham.

Results

We observed markedly disparate transcriptional signatures within the microglia of young-adult and aged mice at baseline which then corresponded to disparate transcriptional responses to TBI (Figure 2). This remarkable differentiation resulted from a unique population identified in microglia of aged mice (cluster 4 &10) and another unique population identified in microglia of young-adult mice (e.g., cluster 2). (Fig. 3). Microglia from aged TBI mice had enriched neuroinflammatory and immune responses (e.g., Th1 and Th2 activation) compared to young TBI mice and their shams. Enriched anti-inflammatory response (e.g., IL-10) in acute TBI was more predominant in aged TBI mice than young counterparts (Figure 4). Just as intriguing is that aged TBI mice had enriched disease-associated-microglia (DAM), a microglial profile implicated in Alzheimer’s Diseases (AD), reflecting the epidemiologic link between TBI and AD (Figure 4).



Aged Sham	Aged TBI	Young Sham	Young TBI
Cluster 0	Cluster 4	Cluster 1	Cluster 2
	Cluster 10		Cluster 7
			Cluster 11

Figure 3. Top: donut charts showing the proportions of different clusters. **Bottom**: table indicating the enriched cluster for individual group. Differentially expressed genes (DEGs) from enriched cluster underwent further analyses in IPA.

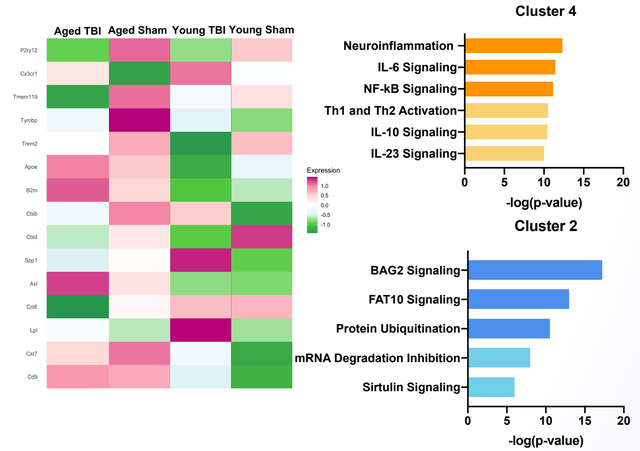


Figure 4. Left: heatmap showing DAM genes differentially expressed between aged TBI versus sham and young TBI versus sham. **Right**: bar graphs indicating the enriched pathways from IPA for unique clusters within aged TBI and young TBI groups.

Conclusions

We hypothesized that there are age-dependent transcription responses within the microglia of young-adult mice as compared to aged mice after TBI. We found that aged microglia adopt a unique phenotype with enriched inflammatory and immune responses. These changes corresponded to a disease-associated phenotype found within the microglia during onset and progression of Alzheimer’s disease. Young-adult mice, on the other hand, upregulated transcripts consistent with a maintenance response to injury. These data suggest that the molecular mechanisms of injury are different between young and aged subjects.

Taken together, our initial findings revealed that microglial responses might account for the age-effect on TBI outcomes. To fully elucidate age-effect, studies are ongoing to determine the molecular alterations at chronic time points (e.g., four months post injury). Meanwhile, we urge that age should be an a priori consideration in future trial design and clinical studies examining the treatment of TBI.

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