Microglia Modulates the Age-effect on Acute TBI Outcomes

Zhangyong Chen1,2, Mecca B.A.R. Islam3, Madeline E. Timken1, Booker T Davis IV1, Steven J. Schwust1

1 Department of Surgery, Division of Trauma and Critical Care, Northwestern University Feinberg School of Medicine, Chicago IL
2 Driskill Graduate Program in Life Science, Northwestern University Feinberg School of Medicine, Chicago IL

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. 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 - 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 fluorescence-activated cell sorting. We used single-cell RNA-seq 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 transcriptional responses within the microglia. Thus, we urge that age should be a priori consideration in the future treatment of TBI.

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

- We utilized single-cell RNA-seq (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/CD11b+ and separated by fluorescence-activated cell (FAC) sorting. Libraries were prepared using the 10x Genomics Chromium Single Cell 3′ Reagent Kits and sequenced on an 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 Satij Lab Laboratory Website (https://satijalab.org/seurat/itex.html).
- Hypogeometric 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-old adult B6 mice 8 hours post TBI versus sham and aged B6 mice 8 hours post TBI versus sham.

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

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

Methods

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) and another unique population identified in microglia of young-adult mice (Fig. 1). Microglia from control TBI mice had enhanced neuroinflammatory and immune responses (e.g., TNF and Th2 activation) compared to young sham mice. Enriched anti-inflammatory response (e.g., IL-10) in acute TBI was more predominant in aged TBI mice than young controls (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).

Conclusions

We hypothesized that there are age-dependent transcriptional 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, unexpectedly transcriptional 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.

Bibliography