Jiaqi Ma (Jiaqi), 1007957686
Dr. Yuzwa, BCB330Y1
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BCB330 Proposal

Impact of white matter injury in mice developing brain at single cell level & comparison to human data based on scRNA-seq

1. Introduction

According to World Health Organization, the rate of preterm birth (born before 37 weeks of pregnancy) was 4~16% in 2020 and 25~50% of preterm infants were reported to have neurodevelopmental deficient[12]. Among survivors, white matter injury (WMI) was found to be the major type of brain injury with symptoms ranging from cognitive to behavioral deficient, and in server cases, direct cause of mortality[11].

Clinically, with the progression of neonatal health care, the pathologic form of WMI changes from severe necrosis, cystic lesion to milder diffuse non-necrotic lesion which further categorized as diffuse white matter injury (dWMI)[4]. By contrast, the cortical gray matter impairment is relatively insignificant and suspect to be secondary impairment by WMI[12].

2. Literature review

The oligodendrocyte lineage development and myelination have been identified as the susceptible target of WMI and heavily studied[1,4,11,12]. In human, the vulnerable perinatal stage was identified between 23~32 postconceptional week (PCW), revealed by increased anti-myelin IHS signal at this stage[3]. Late oligodendrocyte progenitor, or called pre-oligodendrocyte in some literature (preOL), was identified as the predominant OL population during previously revealed vulnerable stage, thus suspect to be a potential target[3].

Subsequently, in terms of pathology, hypoxia-ischemia were found to be particularly triggering damage to preOL[2]. At cellular level, preOL was found to be developmentally arrested in dWMI. Oligo progenitor cell successfully expands preOL population, but preOL failed to further differentiate and mature to proceed myelination[4].

Despite numerous progressions, the underlying molecular mechanism still remains unclear and thus treatment is limited[8]. Combined with the lack of human pathologic sample, scientist was driven to develop many mammalian model. Similar to the clinical change from WMI to dWMI, developed mammalian models also alter their aim from reproduce sever cystic necrosis to non-cystic lesion. Most models share some comment manipulation including hypoxia and ischemia, but differ in ways and timing of manipulation.

The most common choice of model organism is *Mus musculus*, and the most common manipulation is 8% oxygen with unilateral common carotid artery ligation at post-natal day 7 (P7), also called Rice-Vannucci model developed in 1981[10]. The problem nowadays with Vannucci model is it usually generates severe necrosis, whereas clinically diffuse non-necrotic lesion is more prevalent. Thus, researchers were trying milder manipulation like ischemia/hypoxia only[13] or injecting lipopolysaccharide (LPS) to activate immune system instead of ischemia[9].

Regarding bioinformatics, the rapid advancement of next-generation sequencing has enabled researchers to extract specific information of their interest from complex biological systems. At transcriptome level, single-cell RNA sequencing (scRNA-seq) can identify cell lineage from population, reveal marker genes, and imply cell state based on differential gene expression. A typical workflow for scRNA-seq analysis including quality control, normalization, scaling, dimension reduction, and clustering[7].

3. Objective

1) Identify and analyze different cell type in injured mice dWMI model

- Based on known marker gene, identify cluster cell type
- Identify novel/missing cluster in injury group, and provide hypothesis based on differentially expressed gene
- Compare consistent cluster in control & injury group

2) Compare mice and human control group

- Draw interpretation from common/differentially expressed gene across cell types.
- Investigate finding from mice model in human

4. Research Plan

1) Research question

Which cell types are affected in mice dWMI, what is the impact, and what are the differences and similarity between mice and human perinatal stage?

2) Data sets

a) Mice dWMI model scRNA-seq from Yuzwa lab (Unpublished):

This data set utilizes a novel model suggested by Renz, P et al, 2022[9]. LPS (concentration: 0.2 mg/ml) was injected into P2 mice (Weight scale: 2 mg/kg). Followed by 6 hours resting, hypoxia (8% O_2) treated under 34ºC for a duration of 25 min. Control group was injecting saline without subsequent hypoxia treatment. Brain tissue was collected at P11 followed by scRNA-Seq.

b) Rice-Vannucci model snRNA-seq[13]:

This data set contain four groups of data, including hypoxia + ischemia, hypoxia only, ischemia only, and shame control. Slightly adjustment was made compared with original Rice-Vannucci model: resting time between ischemia and hypoxia was reduced from 4 hours to 2 hours, hypoxia duration was reduced from 3.5 hours to 1 hour. Brain tissue was collected at P14 followed by snRNA-Seq.

c) Human single nucleus multiome data[14]:

This data set contain data from four different sources, first, second, and third trimester and early postnatal without any manipulation. Tissue from each development stage underwent nuclei isolation, RNA-seq, and ATAC-seq. This research will focus on the third-trimester RNA-seq data since it aligns with the window of dWMI and is most comparable to previous RNA-seq data.

3) Method

R (4.5.0) and R studio (2025.05.0+496) are utilized for data analysis and visualization. Subsequent analysis is conducted by Seurat (V5) R package[5]. Quality control is based on mitochondrial gene percentage and number of gene count to eliminate low quality/dying/multiplet cell. Log-Normalization then performed to address technical and biological variability. Then scaling (linear transform) to make the mean of expression of each gene to be 0 and variance to be 1, followed by principal component analysis (PCA) to determine the proper dimensionality. Lastly, cluster and run Uniform Manifold Approximation and Projection (UMAP) to visualize clusters. Gene ontology analysis is conducted by ClusterProfiler (4.16.0) R

package[15]. Cluster identity is identified by the combinatory result of known marker genes of each well-studied cell type, differential gene expression of each cluster, and gene ontology. Code written for the analysis is available on Github (https://github.com/DDMMMAA/scRNA_Seq_Scott_Lab).

5. References

1. Back S. A. (2017). White matter injury in the preterm infant: pathology and mechanisms. *Acta neuropathologica*, *134*(3), 331–349. https://doi.org/10.1007/s00401-017-1718-6
2. Back, S. A., Han, B. H., Luo, N. L., Chricton, C. A., Xanthoudakis, S., Tam, J., Arvin, K. L., & Holtzman, D. M. (2002). Selective vulnerability of late oligodendrocyte progenitors to hypoxia-ischemia. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *22*(2), 455–463. https://doi.org/10.1523/JNEUROSCI.22-02-00455.2002
3. Back, S. A., Luo, N. L., Borenstein, N. S., Levine, J. M., Volpe, J. J., & Kinney, H. C. (2001). Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *21*(4), 1302–1312. https://doi.org/10.1523/JNEUROSCI.21-04-01302.2001
4. Buser, J. R., Maire, J., Riddle, A., Gong, X., Nguyen, T., Nelson, K., Luo, N. L., Ren, J., Struve, J., Sherman, L. S., Miller, S. P., Chau, V., Hendson, G., Ballabh, P., Grafe, M. R., & Back, S. A. (2012). Arrested preoligodendrocyte maturation contributes to myelination failure in premature infants. *Annals of neurology*, *71*(1), 93–109. https://doi.org/10.1002/ana.22627

5. Hao, Y., Stuart, T., Kowalski, M. H., Choudhary, S., Hoffman, P., Hartman, A., Srivastava, A., Molla, G., Madad, S., Fernandez-Granda, C., & Satija, R. (2024). Dictionary learning for integrative, multimodal and scalable single-cell analysis. *Nature biotechnology*, *42*(2), 293–304. https://doi.org/10.1038/s41587-023-01767-y
6. Harkins, D., Ali, S., Tockovska, T., Ciganovic, S., Casasbuenas, D. L., Watanabe, S., Ouzikov, S., & Yuzwa, S. A. (2025). Clonal lineage tracing and transcriptomics of cortical progenitor populations reveal maintenance of differentiation potential. *Stem cell reports*, *20*(3), 102418. https://doi.org/10.1016/j.stemcr.2025.102418
7. Hwang, B., Lee, J. H., & Bang, D. (2018). Single-cell RNA sequencing technologies and bioinformatics pipelines. *Experimental & molecular medicine*, *50*(8), 1–14. https://doi.org/10.1038/s12276-018-0071-8
8. Kaindl, A. M., Favrais, G., & Gressens, P. (2009). Molecular mechanisms involved in injury to the preterm brain. *Journal of child neurology*, *24*(9), 1112–1118. https://doi.org/10.1177/0883073809337920
9. Renz, P., Schoeberlein, A., Haesler, V., Maragkou, T., Surbek, D., & Brosius Lutz, A. (2022). A Novel Murine Multi-Hit Model of Perinatal Acute Diffuse White Matter Injury Recapitulates Major Features of Human Disease. *Biomedicines*, *10*(11), 2810. https://doi.org/10.3390/biomedicines10112810
10. Rice, J. E., 3rd, Vannucci, R. C., & Brierley, J. B. (1981). The influence of immaturity on hypoxic-ischemic brain damage in the rat. *Annals of neurology*, *9*(2), 131–141. https://doi.org/10.1002/ana.410090206

11. Volpe J. J. (2009). Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *The Lancet. Neurology*, *8*(1), 110–124. https://doi.org/10.1016/S1474-4422(08)70294-1

12. Volpe J. J. (2019). Dysmaturation of Premature Brain: Importance, Cellular Mechanisms, and Potential Interventions. *Pediatric neurology*, *95*, 42–66. https://doi.org/10.1016/j.pediatrneurol.2019.02.016

13. Wakui, N., Shimbo, T., Hanawa, M., Kitayama, T., Yamamoto, Y., Ouchi, Y., Saga, K., Okada, A., Mimura, K., Tamai, K., & Endo, M. (2025). High-resolution transcriptome analysis on a mouse model of neonatal hypoxic-ischemic encephalopathy using single-nucleus RNA-seq. *Biochemistry and biophysics reports*, *42*, 102026. https://doi.org/10.1016/j.bbrep.2025.102026

14. Wang, L., Wang, C., Moriano, J. A., Chen, S., Zuo, G., Cebrián-Silla, A., Zhang, S., Mukhtar, T., Wang, S., Song, M., de Oliveira, L. G., Bi, Q., Augustin, J. J., Ge, X., Paredes, M. F., Huang, E. J., Alvarez-Buylla, A., Duan, X., Li, J., & Kriegstein, A. R. (2024). Molecular and cellular dynamics of the developing human neocortex at single-cell resolution. *bioRxiv : the preprint server for biology*, 2024.01.16.575956. https://doi.org/10.1101/2024.01.16.575956

15. Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L., Fu, X., Liu, S., Bo, X., & Yu, G. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation (Cambridge (Mass.))*, *2*(3), 100141. https://doi.org/10.1016/j.xinn.2021.100141