Using Human Pluripotent Stem Cell-Derived Microglia as Models for Neurological Disease Research

Jeanne Chan[1], Eloi Mercier[1], Mark Hills[1], Adam Anonuevo[1], Leon Chew[1], Melanie Kardel[1], Allen C. Eaves[1,2], Sharon A. Louis[1], and Erin Knock[1]

[1]STEMCELL Technologies Inc., Vancouver BC, Canada; [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver BC, Canada

INTRODUCTION

Microglia play a critical role in the development and progression of neurological disease, thus human pluripotent stem cell (hPSC)-derived microglia can be a useful model in these studies. hPSC-derived microglia derived from the STEMdiff™ Microglia Culture System can phagocytose disease-relevant peptides such as human fluorescent beta-amyloid peptide (1-42). These cells integrate into cerebral organoids upon co-culture and display an activated morphological change in response to a needle-stick injury. Further, transcriptomics data show that the hPSC-derived microglia express many genes commonly associated with disease risk. These data demonstrate that the hPSC-derived microglia can be a useful model for diseases with a neuroinflammatory component.

METHODS

Microglia Differentiation: hPSCs maintained in mTeSR™1 were differentiated into CD43-expressing hematopoietic progenitor cells (HPCs) using STEMdiff™ Hematopoietic Kit (STEMCELL Technologies 05310) for 12 days. On day 12, the HPCs were differentiated using the STEMdiff™ Microglia Culture System (STEMCELL Technologies 100-0019 and 100-0020) for 28 - 34 days.

Phagocytosis Assay: Functional characterization was performed using 5 µg/mL Human Beta-Amyloid (1-42) HiLyte™ Fluor 555- labeled (Anaspec AS-60480-01) and 28-day-old hPSC-derived microglia over a one-week period. The cultures were imaged with a Leica SP8 confocal microscope.

Microglia and Cerebral Organoid Co-Culture: 2.50 x 10^5 28-day-old hPSC-derived microglia were added to a well containing a single 200-day-old organoid produced using STEMdiff™ Dorsal Forebrain Organoid Kit in STEMdiff™ Neural Organoid Maintenance Medium. A needle-stick injury was inflicted on the co-culture with a 25G needle on day 7. The organoids were analyzed by immunocytochemistry for IBA1 and MAP2 expression after 10 days in culture.

RNA Sequencing: 28-day-old hPSC-derived microglia RNA was extracted using the RNeasy Mini Kit (Qiagen 74104) as directed. A 50-base-pair paired end library was run on an Illumina NextSeq at 20 million reads per sample. Raw data were aligned to the human transcriptome using Salmon and differential expression was determined using DESeq2. Several published data sets were included in the analysis: GSE89189, GSE117829, GSE85839, GSE97744, GSE125872, GSE99074, GSE117040, and GSE36952. Batch correction was performed to compare data sets using LIMMA.

RESULTS

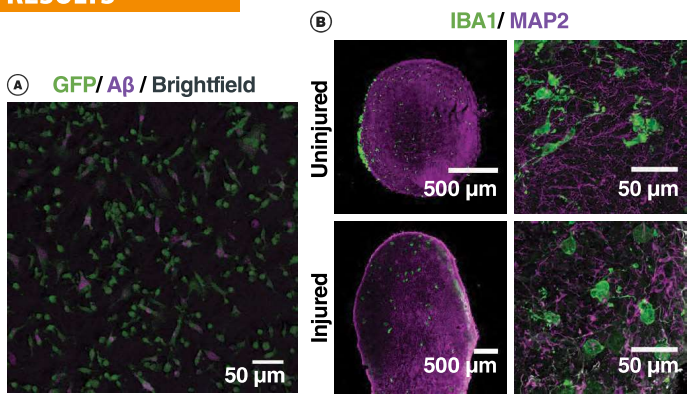

FIGURE 1. hPSC-Derived Microglia Respond to Pro-Inflammatory Stimuli.

(A) A representative image of a time course in which **labeled Aβ (1-42) fragments** were incubated at 37°C with **GFP-labeled hPSC-derived microglia** for 1 week. The microglia were observed to phagocytose the Aβ fragments after 1 day. Scale bar = 50 µm. **(B)** Representative immunocytochemistry images of **IBA1**-expressing hPSC-derived microglia co-cultured with **MAP2**-positive dorsal forebrain organoids. The microglia display an unactivated morphology with extended processes (top panel) and after injury, the microglia take an amoeboid-like morphology (bottom panel). Scale bars = 500 µm (left) and 50 µm (right).

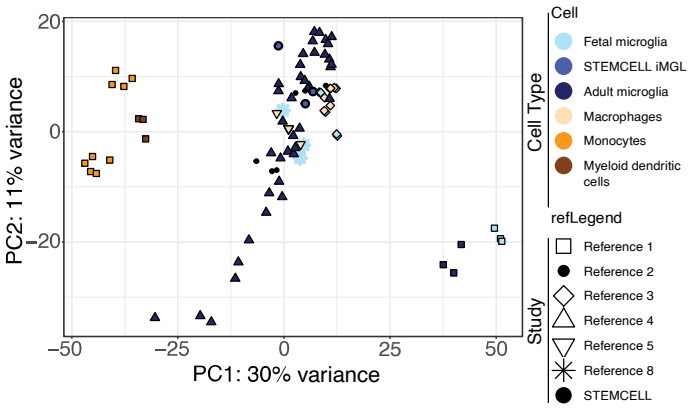

FIGURE 2. Cells from the STEMdiff™ Microglia Culture System are Transcriptionally Similar to Several Published Data Sets for Primary Microglia.

Principal Component Analysis plot comparing RNA-seq profiles from hPSC-derived microglia (iMGL), monocyte, macrophage, dendritic cell, and fetal and adult microglia (MGL) data sets from several publications. The hPSC-derived microglia and most primary microglia data sets cluster together in the center and apart from the monocytes and myeloid dendritic cell types.

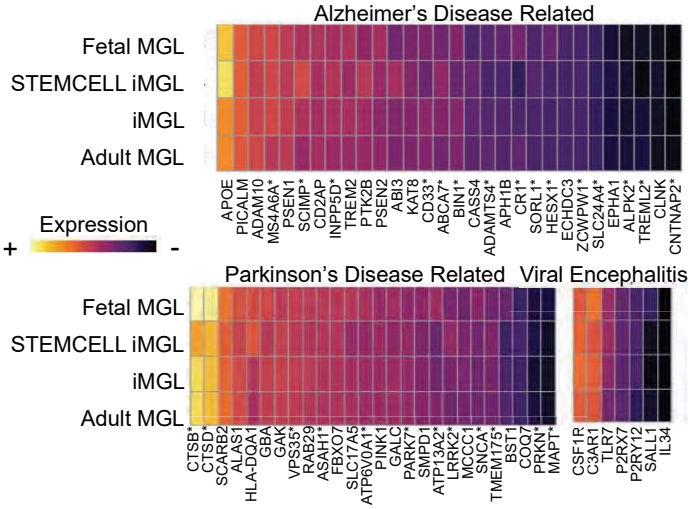

FIGURE 3. Cells from the STEMdiff™ Microglia Culture System Express Disease-Relevant Genes Similar to Other Published hPSC-Derived Microglia.

Heat map of absolute expression levels for select genes associated with Alzheimer's disease, Parkinson's disease, and viral encephalitis expressed in hPSC-derived microglia (iMGL) and primary microglia (MGL). The expression of these genes did not vary much between primary and hPSC-derived microglia. Genes marked by * are those which are significantly different between STEMCELL's hPSC-derived microglia and any of the other 3 groups by differential gene expression analysis (DEseq2, adjusted p<0.05).

Summary

hPSC-derived microglia respond as expected to pro-inflammatory stimuli and express genes relevant for diseases with a strong neuroinflammatory component

References

1. Abud EM et al. (2017) Neuron 94(2): 278–93.e9.
2. Beyer M et al. (2017) PLoS One 7(9): e45466.
3. Douvaras P et al. (2017) Stem Cell Reports 8(6): 1516–24.
4. Galatro TF et al. (2017) Nat Neurosci 20(8): 1162–71.
5. Gerrick KY et al. (2018) PLoS One 13(12): e0208602.
6. Grubman A et al. (2020) Stem Cell Reports 14(6): 1018–32.
7. McQuade A et al. (2018) Mol Neurodegener 13(1): 67.
8. Muffat J et al. (2016) Nat Med 22(11): 1358–67.