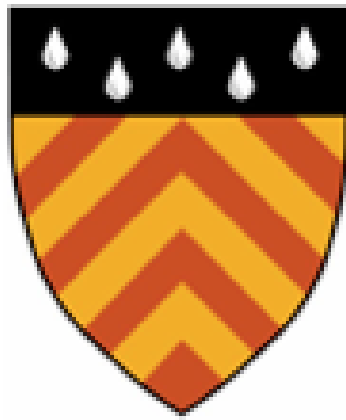


The Transcriptional Profile of Microglia: From Brain to Dish



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Clare Hall

December 2019

University of Cambridge

This thesis is submitted for the degree of Doctor of Philosophy

Declaration of originality

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text. It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

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Fiona Calvert

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Microglia are the tissue resident macrophages of the central nervous system (CNS) and multiple lines of evidence indicate that microglia are a pathogenic cell type in Alzheimer's disease (AD). It is important to understand the transcriptional profiles of microglia, both from primary human cells and the *in-vitro* model systems used to study the cells at scale. In this thesis, I aim to build on previous small-scale studies of primary microglia and *in-vitro* model systems to answer three major questions: **1.** Can transcriptional data from fresh, primary human microglia be used to identify novel subpopulations of cells and understand how clinical phenotypes influence gene expression? **2.** How accurately do current simple *in-vitro* model systems of human microglia capture the profile of primary human cells? **3.** Do more complex model systems move cultured cells further along a trajectory towards the primary cell type?

I have utilised RNA-sequencing technology to build the most comprehensive transcriptional profile of primary human microglia to date, from over 100 neurosurgical patients. Using single-cell sequencing I have demonstrated that clinical pathology, particularly major trauma, causes specific gene expression changes within microglial transcriptomes. I have then shown that *in-vitro* models of primary microglia have significantly reduced expression of key marker genes and transcription factors, such as *P2RY12* and *SALL1*, when compared to primary cells. Using gene-set enrichment analysis tools, I have shown that many of the genes with higher expression in primary cells can be linked to neuronal processes such as CNS myelination. Data from the third chapter of this thesis identified the CNS environment as a major stimulating factor in the gene expression profile of primary microglia. Therefore, I used single cell analysis to understand how culturing stem cell derived microglia in the presence of neurons could move *in-vitro* systems closer towards the primary cell type. In summary, the work in this thesis has demonstrated that microglial transcriptomes are constantly reacting to stimuli within the local CNS environment, both to maintain their unique gene expression profiles and to respond to clinical conditions. I have also shown that current *in-vitro* model systems do not fully capture this transcriptional profile which largely appears to be driven by environmental stimuli within the CNS.

Acknowledgments

I sat staring at this page for a little while before writing this, I was unsure of how to put into words the thanks and gratitude I have for all the people who have helped me get to the point of completing this. I also have a tendency to overdo the sappy and felt pressured to write something witty and lighthearted. What follows, much like my PhD, I'm sure will be different from what I expected and planned but something that I will be proud of nonetheless.

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Abbreviations

Alzheimer's disease	AD
Blood brain barrier	BBB
Central nervous system	CNS
Colony stimulating factor 1 receptor	CSF-1R
Embryoid body	EB
Expression quantitative trait loci	eQTL
Fluorescence-activated cell sorting	FACS
Genome-wide association studies	GWAS
Induced pluripotent stem cells	iPSCs
Knockout	KO
Late onset Alzheimer's disease	LOAD
Log fold change	LFC
Monocyte derived macrophages	MDMs
Multiple sclerosis	MS
Nuclease free water	NFW
Peripheral Blood Mononuclear Cells	PBMCs
Principal components analysis	PCA
Polymerase chain reaction	PCR
Quality control	QC
Quantile normalisation	QN
Single cell RNA-seq	scRNA-seq
Single nucleotide polymorphism	SNP
Traumatic brain injury	TBI
Transcription factor	TF
Transcripts per million	TPM
Uniform Manifold Approximation and Projection	UMAP
Variance stabilisation transformation	VST
Yolk sac	YS

