The Transcriptional Profile of Microglia: From Brain to Dish

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December 2019

University of Cambridge

This thesis is submitted for the degree of Doctor of Philosophy

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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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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 soppy 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.

First of all, I have to acknowledge Dan Gaffney, my ever wonderful and patient supervisor. I think this PhD has been a learning curve for both of us but I cannot stress enough how vital a part you have played in my journey here. You have pushed and challenged me in ways I couldn't have imagined when I started over four years ago. Throughout it all, you have also provided constant support and encouragement and have never made me feel like I couldn't do this. Thank you for taking a student who didn't even know the terminal existed and only knew how to perform a t-test and shaping me into an R-loving scientist with an annoying obsession for correct statistics. I would also like to acknowledge the wonderful lab you have built, a group of people who have been such a huge part of my PhD. To Andy, who acted as my supervisor within the lab and is truly one of the most remarkable scientists I have ever met. You made me a better scientist and opened my eyes to the wonderful world of moths and orchids. I hope you know that the Gaffney lab is only what it is, in part, because of you. To Julie and Clara, who I could not have survived the last couple of months without, thank you for listening to my gripes and moaning and for always giving the best life advice. To everyone else in the Gaffney lab (Natsuhiko, Nikos, Beata, Maria P, Maria I and Gerda) thank you for providing so many laughs and thoughtful discussions over lunch and coffee breaks. It has been a pleasure to work alongside you all and I hope our GIF-filled slack channels will live on forever!

To everyone I have had the pleasure of working with in these four years outside of the PhD - thank you for providing me with the most wonderful distractions when the PhD took its toll. To the members of staff at Clare Hall, who helped us put on an event that is one of my proudest achievements. The May Ball was a shining light of my four years. To everyone I have met through the Story Collider, the producers and the storytellers, you are the most inspiring people I have ever met. You have changed the way I view science and have opened my eyes to a whole new wonderful world. Particularly to Erin, Liz and Steve - you have taught me so much in such a short space of time and I honestly feel privileged every day that I get to work with such ridiculously amazing people.

I could not have gotten to the point of finishing this PhD without my personal support network. To Lindsay, who I met at the Sanger interviews and knew we would be friends instantly - THANK YOU. You quickly became one of my best friends and I will always be grateful to have someone to cry in the toilets with. I write this full of pride that we both made it through, I wouldn't be here without you. To all my friends outside the PhD, both old and new: Emily, Becky, Emma, Madi, Guy, Luke, Sarah and Christy - thank you for providing wonderful relief from this process and filling my life with joy and excitement. My parents have always been, and I'm sure will continue to be, my biggest cheerleaders. I owe everything I have achieved to the strength and encouragement you have given me. I am so proud to be your daughter and don't know how to ever thank you for all you have done for me. I promise to only make you read this small part of my PhD and will not enforce proof-reading duties on you, even though I know you would if I asked. I love you both so much. Finally, the biggest acknowledgment of all. To Will, my long suffering partner who has been by my side for the majority of this PhD. You have had to listen to me complain about every failed experiment, every bug in my code and every frustration. You have seen me at my worst and provided me with every bit of support I need to pull myself out of those dark spaces. You made me laugh every day and have shown me how to not take life too seriously, something I desperately needed. You have been my rock, my escape and my source of happiness throughout every up and down of this PhD. I cannot thank you enough for being part of my life and for learning what microglia are for me.

Lindsay - we had many discussions about this very point so I include this as an acknowledgement of our journey. A large part of this acknowledgement goes to me, this thesis will forever be a reminder of what I can achieve even when I don't think I can.

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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