Single cell biology of the human hypothalamus in obesity and hypertension

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Project outline

Background: Obesity and hypertension, which frequently co-exist in individuals, represent major risk factors for multiple conditions from heart disease to dementia as well as leading to shortened life expectancy [PMID: 35711612]. They are modifiable risk factors, so with the right treatments could have huge impacts on the burden of disease. The hypothalamus, a highly heterogenous and complex structure, is the central regulator of energy and body homeostasis. Particularly relevant nuclei are the paraventricular nucleus, suprachiasmatic nucleus and the medial hypothalamic nuclei [PMID: 34266604, 28592656]. There is growing evidence of cell-type specific dysfunction and dysregulated cell-cell crosstalk within these nuclei underlying obesity and hypertension. Single cell approaches provide effective means to understand cell-type specific molecular mechanisms and cell-cell interactions in the healthy human hypothalamus [Tadross et al., Human HYPOMAP bioRXiv 2023]. However, it remains unknown how these hypothalamic cellular networks are affected by extreme BMI and hypertension, limiting the effective exploration of novel therapeutic strategies.

We developed a novel snRNA-seq method (manuscript in prep) to extract and sequence both vascular (endothelial cells, pericytes) and parenchymal (neurons, glia) from fresh frozen human post-mortem brain with high efficiency. Working with NNRCO, we have already successfully applied this technique to human prefrontal cortex from 44 patients with diabetes, hypertension or both. This has revealed that brain vascular cells are relatively protected in diabetes but specific vascular cell types are substantially altered in hypertension. For this proposal, we will aim to use our snRNA-seq approach to dissect pathogenic processes in the hypothalamus for obesity and hypertension.

Hypothesis: We propose cell-type specific processes and inter-cellular signalling within the hypothalamus are driving mechanisms in obesity and hypertension and accounts for their frequent co-morbidity.

Aims: Our overall goal is to perform single cell analysis of the human hypothalamus to identify the cell population and associated gene networks altered in obesity and/or hypertension. Our specific aims are:

1. Perform single nuclei isolation and snRNA-seq on human fresh frozen hypothalamus from 200 donors with a range of body mass index (BMI); with or without hypertension.
2. Identify key cell types in the hypothalamus and assess for changes in cell-type composition, new cell states and differentially expressed genes associated with BMI and hypertension. This analysis will also control for age and account for sex dimorphism
3. Validate changes in genes and corresponding proteins using single molecule in situ hybridisation (RNAscope) and immunocytochemistry on human post-mortem hypothalamic tissue sections; and functional validation using human induced pluripotent stem cell (iPSC) models.

Research Methodology

Year 1: The Oxford Brain Bank (led by Laura Parkkinen) has a unique collection of donor post-mortem fresh frozen tissue which do not have significant neurodegeneration and therefore represent ideal starting material for this project. De-identified medical information is available to establish BMI, hypertension status and other co-morbidities. Using protocols developed in the Cader lab, 200 donor fresh frozen hypothalamus will be subject to single nuclei dissociation, followed by fixation and library preparation using Parse Split-Seq [PMID: 29545511] and then Illumina sequencing. This was the method previously employed in the NN pilot study that generated high quality snRNAseq data from 44 diabetes/hypertension donors. All donors will have genome wide-SNP typing.

Year 2: Single nuclei data will be subjected to quality control processes, sequence annotation/alignment and analysis pipelines in the Cader lab and at the NNRCO. Key cell-types will be identified based on marker enrichments and comparison with other single cell datasets. Gene associations with BMI and hypertension will be examined, using regression models that will include age and sex as covariates. WGCNA, NicheNet etc will be used to understand co-correlation networks and cell-cell communication. Therapeutically addressable and novel pathways will be identified and prioritised for validation. Using SNP data, across the large number of donors we will establish cell-type specific e-QTLs which will enable understanding the functional biology of obesity/hypertension GWAS hits.

Year 3: We will validate the key findings from the single cell analysis on human brain hypothalamic tissue slices using in-situ hybridisation (ISH) and immunocytochemisty. (ICC) We have established expertise of using RNAscope for ISH to confirm gene expression changes and a range of ICC protocols with antigen retrieval to confirm gene changes translates into protein changes. These studies will also enable evaluation of the spatial context of the gene/protein changes to confirm the particular nuclei and cell populations that are affected. The most promising targets will then be evaluated in functional assays including iPSC disease models leveraging the expertise in the Cader lab to understand how gene and protein networks alter hypothalamic functional biology.

Supervisor's recent relevant publications:

1. Tsartsalis S,..,**Cader MZ**, Matthews PM. A single nuclear transcriptomic characterisation of impaired angiogenesis and blood brain barrier function in AD. Nature Communications (manuscript accepted)
2. Adriaanse BA,.., **Cader Z**, Zhang B, DeLuca GC. Tuberous Sclerosis Complex-1 (TSC1) contributes to selective neuronal vulnerability in Alzheimer's Disease. Neuropathol Appl Neurobiol. 2023 Apr 5
3. Ng B,.., **Cader ZM**, Lovestone S, Wade-Martins R. Neurons derived from individual early Alzheimer's disease patients reflect their clinical vulnerability. Brain Comm. 2022 Oct21;4(6):fcac267.
4. Pokhilko A, Brezzo G, Handunnetthi L, Heilig R, Lennon R, Smith C, Allan SM, Granata A, Sinha S, Wang T, Markus HS, Naba A, Fischer R, Van Agtmael T, Horsburgh K, **Cader MZ**. Global proteomic analysis of extracellular matrix in mouse and human brain highlights relevance to cerebrovascular disease. J Cereb Blood Flow Metab. 2021 Sep;41(9):2423-2438.
5. Pokhilko A, Handel AE, Curion F, Volpato V, Whiteley ES, Bøstrand S, Newey SE, Akerman CJ, Webber C, Clark MB, Bowden R, **Cader MZ**. Targeted single-cell RNA sequencing of transcription factors enhances the identification of cell types and trajectories. Genome Res. 2021 Jun;31(6):1069-1081.