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Single cell transcriptomics of the cerebral cortex of mice lacking the PRC2 gene *eed*

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Abstract

Objective The Polycomb Repressive Complex 2 (PRC2) regulates neural stem cell behaviour during development of the cerebral cortex, yet how the loss of PRC2 developmentally influences cell identity in the mature brain is poorly defined. Using a mouse model in which the PRC2 gene *Embryonic ectoderm development* (*Eed)* was conditionally deleted from the developing mouse dorsal telencephalon, we performed single nuclei RNA sequencing (snRNA-seq) on the cortical plate of an adult heterozygote *Eed* knockout mouse and an adult homozygote *Eed* knockout mouse compared to a littermate control. This work was part of a larger effort to understand consequences of mutations to PRC2 within the mature brain.

Results Here we provide snRNA-seq data from the cortical plate of an adult heterozygous conditional *Eed* knockout, an adult homozygous conditional *Eed* knockout and an adult control mouse. This data provides insight on how loss of PRC2 function during development affects cell identity in the mature cortex.

Keywords Single nuclei RNA-sequencing (snRNA-seq), Eed, Polycomb repressive complex 2 (PRC2), Cerebral cortex

Objective

Epigenetic modifiers play a crucial role in the development of the brain by regulating neural progenitor identity, differentiation and cell identity post-differentiation. One such epigenetic modifier is the Polycomb Repressive Complex 2 (PRC2). PRC2 functions by methylating lysine 27 on histone H3 (H3K27), which is a repressive epigenetic mark [1]. Studies have demonstrated that PRC2 regulates neural progenitor cell proliferation and differentiation [2–5]. How the absence of this mark during development manifests in the mature brain is, however, not well understood.

The core components of PRC2 include Enhancer of Zeste Homolog 1/2 (EZH1/2), Suppressor of Zeste 12 (SUZ12), Retinoblastoma binding protein 4/7 (RBBP4/7) and Embryonic ectoderm development (EED). Loss-of-function in any of these core components results in a loss of PRC2 function and loss of H3K27 methylation. Here, we used a mouse model in which the key PRC2 gene *Eed* was conditionally knocked out (cKO) from the dorsal telencephalon at embryonic day (E) 9.5, resulting in loss of PRC2 activity [6]. This model was created by crossing $Eed^{fl/fl}$ mice [6, 7] with *Emx1-iCre* mice [8, 9]. We performed snRNA-seq on the whole cortical plate of adult control (CTRL), heterozygous *Eed* knockout (*Eed-cHet*) and homozygous knockout (*Eed-cKO*) mice, allowing for the effects of PRC2 loss-of-function in the cerebral cortex to be investigated. This work was done a part of a larger

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Table 1 Overview of data files/data sets

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
Data set 1 - https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM8504090	CTRL raw data	.tsv.gz .mtx.gz	Gene Expression Omnibus [GSE276683]
Data set 2 - https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM8504091	cHet raw data	.tsv.gz .mtx.gz	Gene Expression Omnibus [GSE276683]
Data set 3 - https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM8504092	cKO raw data	. .tsv.gz .mtx.gz	Gene Expression Omnibus [GSE276683]
Data set 4 - https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE276683	Processed data	.rds	Gene Expression Omnibus [GSE276683]

project investigating the role of PRC2 in the mature cerebral cortex [10].

Data description

This data set is snRNA-seq of the whole cortical plate of a CTRL, *Eed-cHet*, and *Eed-cKO* mouse. This technique provides high detail on gene expression in nuclei isolated from individual cells, allowing the role of EED, and consequently PRC2, to be investigated in specific cell types.

A *Eed/Emx1*-iCre mouse model was in this study [10], which was created by crossing $Eed^{fl/fl}$ mice with *Emx1-iCre* mice. The $Eed^{fl/fl}$ mice were originally obtained from the Jackson Laboratory Stock Center (stock number 022727) [7]. The *Emx1-iCre* mice were obtained from the Queensland Brain Institute [8] and were originally sourced from Kessaris et al. [11]. These mice have been validated previously [6, 7, 9]. This cross produced $Eed^{wt/fl}$; *Emx1*-iCre^{+} mice. These mice were then crossed to $Eed^{fl/fl}$ mice to generate experimental animals, comprising control (CTRL, i.e. $Eed^{fl/fl}$; *Emx1*-iCre^{-}), heterozygous *Eed* knockout (*Eed-cHet*, i.e. $Eed^{wt/fl}$; *Emx1*-iCre^{+}) and homozygous knockout (*Eed-cKO*, i.e. $Eed^{fl/fl}$; *Emx1*-iCre^{+}) mice. One animal was sequenced per genotype; each were 15-week-old females from the same litter. Single nuclei RNA-seq was chosen rather than the more common single cell RNA-seq because it provides a better representation of cells with long processes such as projection neurons, which often get damaged during sample processing.

Mice were euthanised by cervical dislocation as this is a rapid method of euthanasia that minimises distress for the animal. Tissue dissection and single nuclei extraction was performed immediately afterwards (details on extraction method provided on GEO [GSE276683]). The cell suspensions underwent fluorescence activated cell sorting (FACS), using DAPI to filter nuclei from debris and to avoid collection of doublet nuclei. The cDNA library was prepared with the 10x Genomics Chromium platform using a 3' v3.1 kit. Approximately 7000 nuclei per sample were loaded to achieve a target barcoded library of approximately 3500 nuclei per sample. The libraries were then sequenced on a NovaSeq 6000 with a sequencing depth of approximately 78,000-100,000 reads/nuclei.

Remapping to a pre-mRNA genome, barcode processing, gene counting and aggregation was performed using the Cell Ranger 7.0.1 (Table 1, data set 1–3). Following this, the datasets of the three samples were merged and cells with >2% mitochondrial RNA were removed as these are likely to be low-quality cells such as dead or dying cells. Using the Seurat toolkit for single cell genomics (version 4.1.0) [12], the data were normalised with both SCTransform [13] (for cluster calculations) and NormaliseData (for data visualisation). Cells were then clustered with the standard Seurat workflow (RunPCA, RunUMAP, FindNeighbors, FindClusters) (dims=1:30, resolution=0.2). Clusters were identified and labelled based on canonical gene expression. Three small clusters were identified to be cells from subcortical regions - the subiculum and the striatum. These were likely present due to minor errors during dissection and were removed as the aim of dataset is to focus on the cortical plate. Additionally, one small cluster (containing only 55 cells) could not be identified based on gene expression and had low nFeature and nCount values compared to the other clusters. As such, it was determined that these cells were likely unhealthy and they were removed from further analyses. Following quality control, the number of nuclei was 2645 CTRL, 4544 *Eed*-cHet, and 3652 *Eed*-cKO nuclei. This processed data is provided (Table 1, data set 4).

Limitations

One limitation of this work is that only one biological replicate was used per genotype, although this is mitigated by the fact that we sequenced over 2500 individual nuclei per sample. A second limitation related to the conditional approach we employed. By driving *Eed* ablation specifically from neural progenitor cells within the embryonic dorsal telencephalon (using a conditional *Eed* allele crossed to an *Emx1-iCre* driver), other cell types within the mature dorsal telencephalon that are not derived from these progenitor cells would retain PRC2 function. Examples of this includes cortical interneurons,

which are derived from the ventral telencephalon, as well as vascular cells. As such, the population of nuclei we sequenced comprised a mosaic of cells both with, and without, PRC2 function.

Abbreviations

cDNA	Complementary DNA
CTRL	Control
Eed	Embryonic ectoderm development
Eed-cHet	Eed conditional heterozygous knockout
Eed-cKO	Eed conditional homozygous knockout
FACS	Fluorescence activated cell sorting
GEO	Gene expression omnibus
PRC2	Polycomb Repressive Complex 2
snRNA-seq	Single nuclei RNA sequencing

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Author contributions
LC contributed to methodology, investigation, and formal analysis. LH contributed to methodology and supervision. MP contributed to project design, supervision and funding acquisition.

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Data availability
The data described in this Data note can be freely and openly accessed on Gene Expression Omnibus (GEO) under GSE276683. Please see Table 1 and references [10] for details and links to the data.

Declarations

Ethics approval and consent to participate
All animals were used with approval from the University of Queensland Animal Ethics Committee (AEC approval number 2022/AE000397). All work was carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and the University of Queensland's Institutional Biosafety Committee.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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