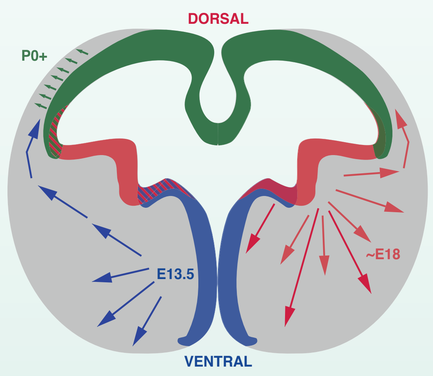
Temporal and developmental dynamics of the oligodendrocyte precursor transcriptome

Darya Vanichkina[123*], Sueli C Marques[4], Ana M Falcao[4], John EJ Rasko[125], Ryan J Taft[36], Goncalo Castelo-Branco[4]

1. Gene and Stem Cell Therapy Program, Centenary Institute, University of Sydney; 2. Sydney Medical School, University of Sydney; 3. Institute for Molecular Bioscience, University of Queensland;
4 Laboratory of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet; 5 Cell & Molecular Therapies, Royal Prince Alfred Hospital; 6 Illumina, San Diego, California

Oligodendrocyte precursor cells (OPCs)

* OPCs are an abundant progenitor cell in the developing and adult brain, constituting 3-8% of total brain cells in adults
* OPCs give rise to oligodendrocytes (OL) - glial cells responsible for myelinating the processes of neurons
* Three waves of OPC generation have been described in mammals: (1) prenatally in the ventral forebrain, (2) early in postnatal development, resulting in myelination of the majority of axons in the nervous system, and (3) a subset continue to develop into OLs throughout all stages of life, involved in myelin remodeling by replacing OLs that have died or by intercalating among existing myelin sheaths
* Functional and physiological differences between OPCs from the brain and spinal cord have been reported, but the molecular basis remains poorly understood



Objectives

1. To purify OPCs from mouse brain and spinal cord at two developmental time points
2. To compare the transcriptome of these cells to understand how transcriptional complexity underpins observed functional differences

Methods

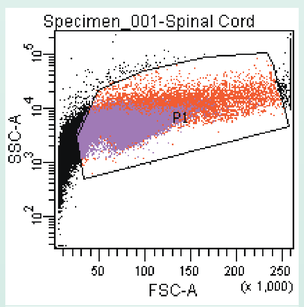
1. Purifying OPCs from mouse brain and spinal cord at two developmental time points

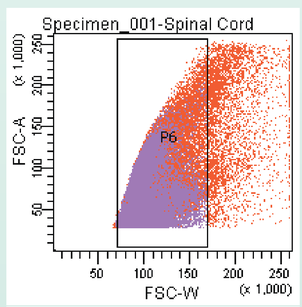
* FACS sorting GFP+ cells from *B6.129S4-Pdgfra*$^{tm11(EGFP)Sor}$*/J* mice from wave 1 (E13.5) and waves 1-2 (P7) of OPC development, from brain (B) and spinal cord (S).

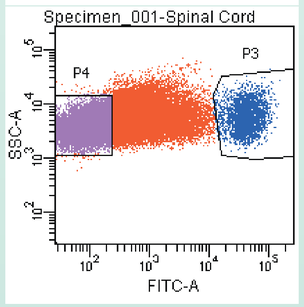
2. Bioinformatic analysis

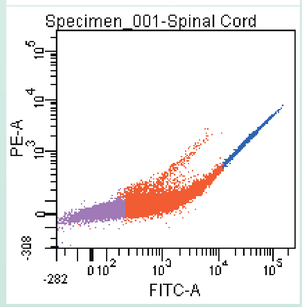
* 150bp PE stranded RNA-seq mapped using STAR with GENCODE M8 as a splice junction reference. Read pairs counted using featureCounts. Differential gene expression assessed with limma voom; variance-stabilizing transformation and PCA analysis using DESeq2. Pathway analysis using IPA & ToppGene.
IRFinder for identifying intron retention.

1. FACS sorting enables purification of OPCs from two distinct developmental stages and CNS regions









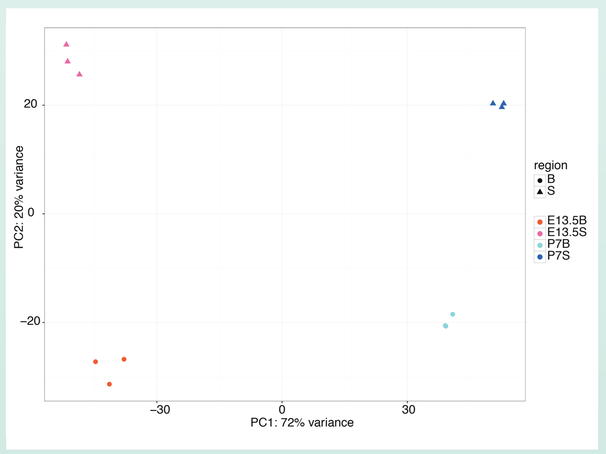
Tube: Spinal Cord

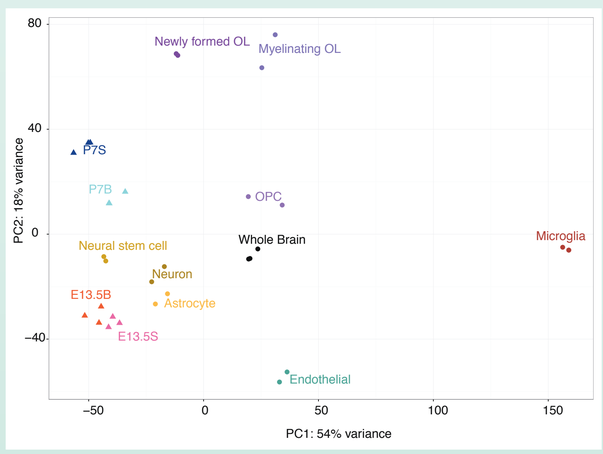
Population	#Events	%Parent	%Total
All Events	122,087	####	100.0
P1	83,338	68.3	68.3
P6	79,307	95.2	65.0
P3	3,000	3.8	2.5
P4	37,040	46.7	30.3

FACS sorting of representative E13.5 spinal cord sample. The gating tree was set as follows: FSC/SSC represents the distribution of cells in the light scatter based on size and intracellular composition, respectively, (P1); SSC/pulse width excludes events that could represent more than 1 cell (P6).

The high GFP expression population (P3) comprised 2.5% of total assessed events, was considered to be OPCs and used for subsequent RNA purification and sequencing.

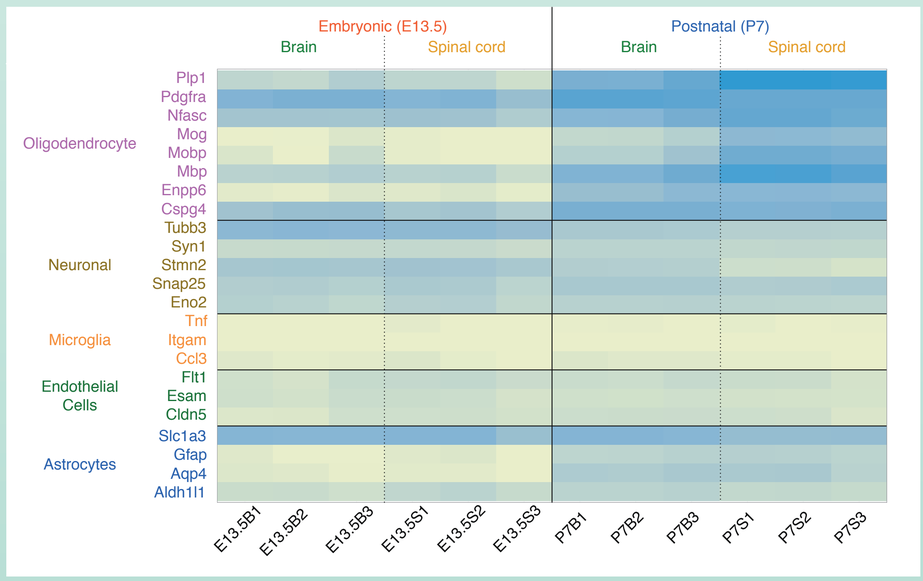
2. PCA analysis reveals that samples cluster based on developmental time point and region, and with similar cell types from a CNS panel



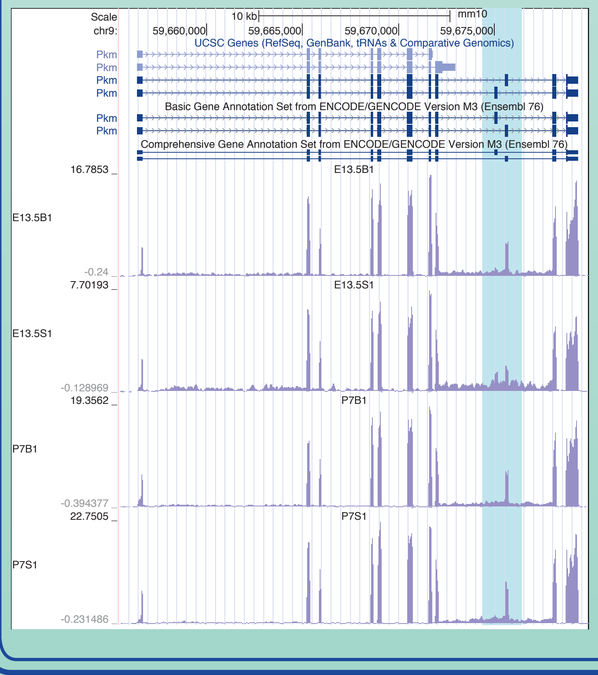


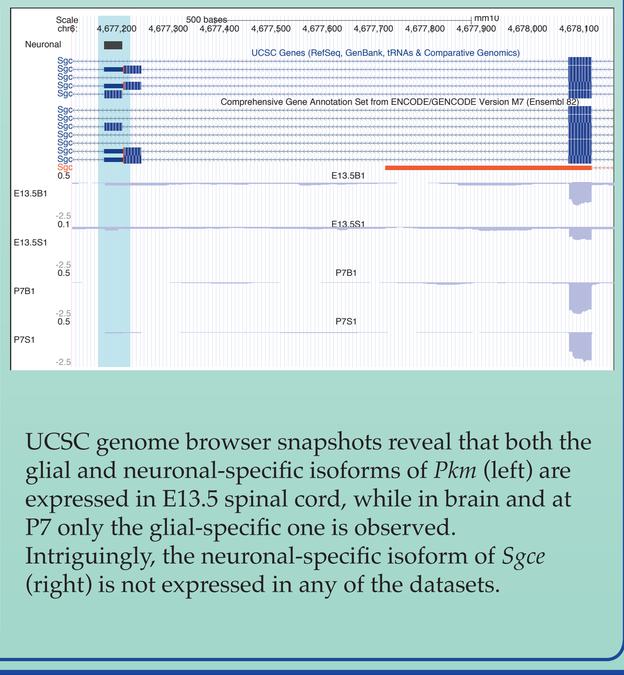
Principal component analysis (PCA) was carried out using the DESeq2 software package with a variance stabilizing transformation carried out without annotation of tissue source ("blind"). All genes were used for variance estimation for OPC samples (left), while for contrasting with tissues the union of the 500 most enriched genes for each pairwise comparison was considered, resulting in a total of ~3700 genes (right; raw data from (X) and (Y).

3. PDGFRA+ cells express significantly more OL markers at P7 than E13.5



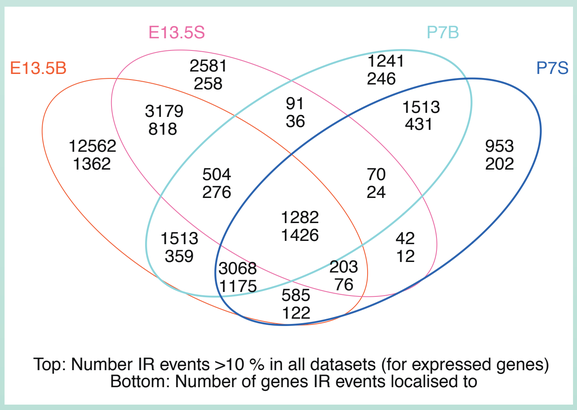
4. Glial-specific splicing isoforms are detected at both time points



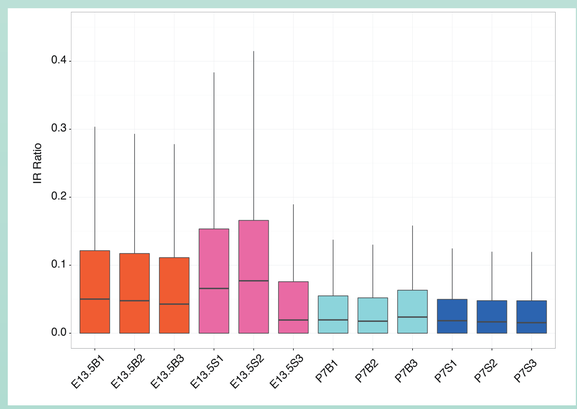


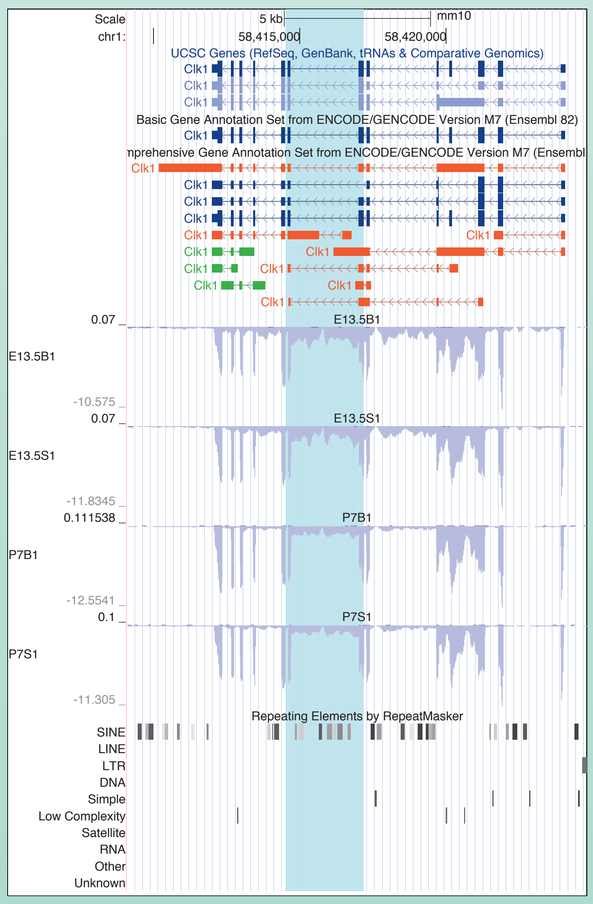
UCSC genome browser snapshots reveal that both the glial and neuronal-specific isoforms of *Pkm* (left) are expressed in E13.5 spinal cord, while in brain and at P7 only the glial-specific one is observed. Intriguingly, the neuronal-specific isoform of *Sgce* (right) is not expressed in any of the datasets.

5. Intron retention (IR) is observed in both embryonic and postnatal OPCs, and affects genes critical for OPC function



Top: Number IR events >10 % in all datasets (for expressed genes)
Bottom: Number of genes IR events localised to





* IRFinder, a sensitive pipeline for detecting IR developed in the Rasko laboratory, was used to assess intron retention

* IR affects a significant number of introns belonging to many distinct genes (top panel)

* Higher levels of IR are detected in embryonic than in postnatal samples (bottom panel)

* Many of these genes are critical for OPC function

Conclusions

1. Oligodendrocyte precursor cells were purified from brain and spinal cord at two developmental time points.

2. Transcriptome analysis revealed that at E13.5 gene expression in PDGFRA+ cells was similar to that of stem cells, while at P7 cells expressing this receptor appeared more committed to the OL lineage .

3. Intron retention is observed in a significant number of genes, across multiple introns. Many of these genes are critical for cellular development or OL function.

4. Ongoing work involves using single cell RNA sequencing to understand whether PDGFRA+ cells at E13.5 are a mixed population, with a large number of neural stem cells and a small proportion of lineage-committed OPCs, or whether all cells that express PDGFRA+, and are hence currently considered to be OPCs, at E13.5 represent a neural stem cell like population and cannot be considered to belong to this cell type.

Contact: d.vanichkina@centenary.org.au