**Human amnion epithelial cell therapy modulates oligodendrocyte heterogeneity in experimental stroke.**

Yeshwanth R Yeraddu¹, David E Whong Zhang¹, Malathi I Dona¹,², Grant R Drummond¹, Christopher G Sobey¹, Maria Jelinic¹,² & T. Michael De Silva¹,

¹Department of Microbiology, Anatomy, Physiology and Pharmacology, Centre for Cardiovascular Biology and Disease Research, La Trobe University1, Melbourne, VIC, Australia. 2Baker Heart and Diabetes Research Institute, Prahran, VIC, Australia.

**Introduction.** Stroke is a leading cause of death and disability worldwide. Current treatment options have a narrow therapeutic window and do not address secondary injury. Human amnion epithelial cells (hAECs) have several properties that make them a promising cell-based therapy. Ischaemic injury leads to loss of brain cells within minutes, but the transcriptional changes that occur during the delayed phase after stroke in response to hAEC therapy are unknown.

**Aim.** To investigate the effects of stroke and hAEC therapy on transcriptional changes in brain cell populations using single-cell transcriptomics.

**Methods.** Male and female C57Bl6 mice (14-18 weeks, *n*=3 per group) underwent sham or photothrombotic stroke surgery targeting the prefrontal cortex and received saline or 1 × 10⁶ hAECs intravenously 24 hours post-stroke. Five weeks after stroke, brains were collected. A 3 mm section from the infarct region was prepared for single-cell RNA sequencing. Data were quality filtered, clustered, and analysed for differential gene expression and gene ontology to identify treatment-associated molecular changes.

**Results.** Single-cell analysis identified 12 brain cell populations. Additionally, we identified multiple oligodendrocyte subclusters in saline- or hAEC-treated mice. hAEC therapy modulated oligodendrocyte transcriptional profiles, with upregulation of genes related to survival, myelination, and neuroprotection, and downregulation of pro-apoptotic and inflammatory pathways. Unique oligodendrocyte subpopulations in the hAEC group displayed enhanced repair signatures and reduced injury markers. Gene ontology analysis supported these findings, suggesting a shift towards a reparative phenotype in oligodendrocytes with hAEC treatment.

**Discussion.** The modulation of oligodendrocyte gene expression by hAEC treatment suggests a potential role in promoting brain repair mechanisms after stroke. Further investigation of additional brain cell populations and their interactions will be important to fully elucidate the therapeutic effects of hAECs.