Deciphering the Molecular, Cellular, and Brain-Level Impacts of Down Syndrome on Astrocytes

Grace Akatsu[1,2], Hiruy Meharena[1,3], and Li-Huei Tsai[1]

1. The Picower Institute for Learning and Memory, MIT, Cambridge, MA | 2. University of Colorado Anschutz, Aurora, CO | 3. School of Biological Sciences, UCSD, San Diego, CA

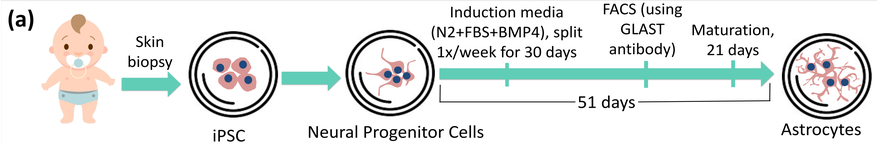
Abstract

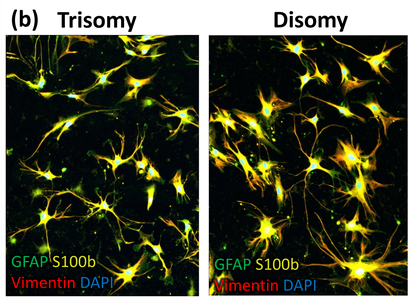
Down syndrome (DS) is a genetic condition caused by triplication of chromosome 21, which frequently results in atypical brain development, learning and memory deficits, and Alzheimer's disease. Astrocytes are non-neuronal central nervous system support cells that play a vital role in processes required for brain function and morphogenesis. A dysfunction in these cells has been observed in neurological disorders such as amyotrophic lateral sclerosis (ALS), epilepsy, and hepatic encephalopathy[1]. In DS, astrocytes have been shown to exhibit higher levels of reactive oxygen species[2], decreased synaptogenic molecule expression[2], and overall greater numbers in the brain[3]. However, our genome-wide transcriptional analysis of iPSC-derived astrocytes revealed additional disrupted biological processes. Using human iPSC-derived astrocytes and a DS mouse model (Ts65Dn), our preliminary data suggests that these cells have a dysfunction in endocytosis, glutamate homeostasis, and angiogenesis, processes involved in learning and memory. Previous DS clinical trials have focused on modulating neuron development and function through avenues such as GABA inhibition or myo-inositol suppression[4]. Our results indicate that astrocytes may be a valuable therapeutic target for treating DS.

Astrocyte Functions

- Angiogenic factor secretion
- Blood-brain barrier regulation
- Glycogen storage (brain energy reserve)
- Cytokine secretion (microglial activation)
- Antioxidant secretion
- Neurotrophic factor secretion
- Extracellular matrix regulation
- Growth factor secretion
- Neurotransmitter uptake and regulation
- Glutamate regulation
- Extracellular ion regulation

iPSC-derived Astrocytes





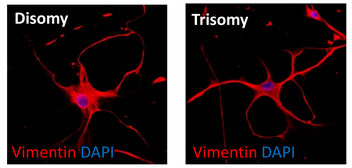


Fig. 1 Astrocytes generated from an isogenic pair of iPSCs[5]. a) *Induction and maturation timeline.* ***b)*** *Staining for GFAP (green), S100b (yellow), vimentin (red), and DAPI (blue).* ***c)*** *Vimentin staining (red) depicting star-like morphology of mature astrocytes.*

Genome-wide Transcription Disruption

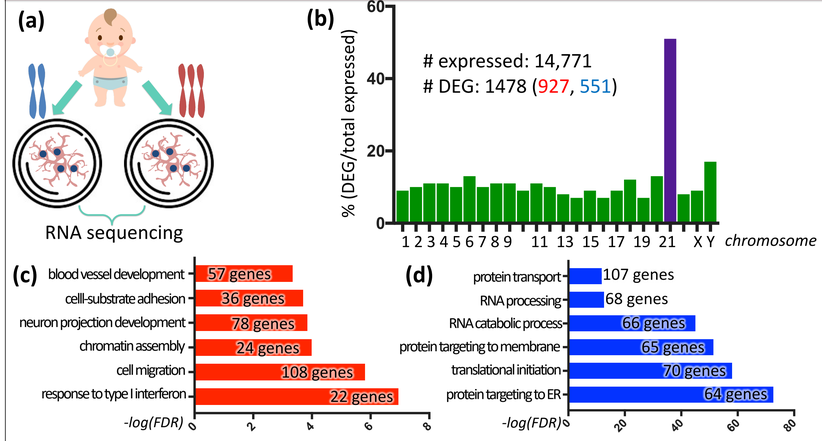
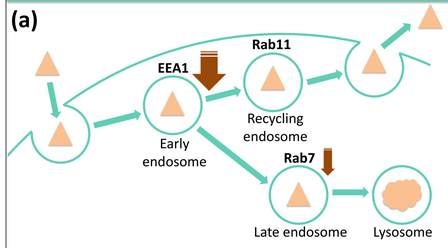
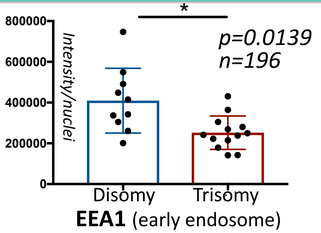
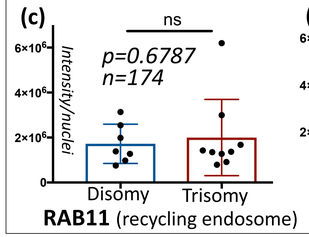


Fig. 2 Impact of DS on the transcriptome. a) *Cartoon schematic of RNA sequencing experimental design.* ***b)*** *Percentage of differentially expressed genes (DEGs) per chromosome.* ***c), d)*** *Gene ontology terms and corresponding false discovery rates (FDR) of upregulated and downregulated DEGs, respectively.*

Decreased Early Endosomes







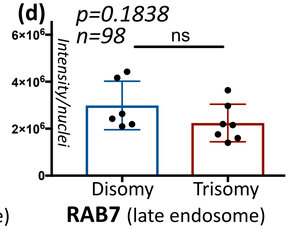


Fig. 3 Impact of DS on endocytosis. a) *Cartoon schematic of the endocytic pathway.* ***b), c), d)*** *Immuno-fluorescence intensity quantification of EEA1, RAB11, and RAB7 staining using Imaris.*

Increased Intracellular Glutamate

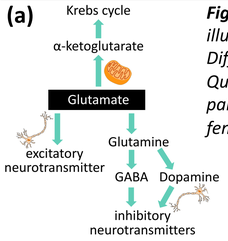
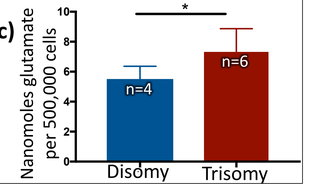
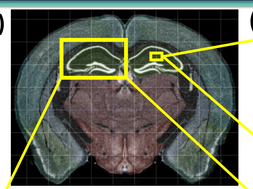


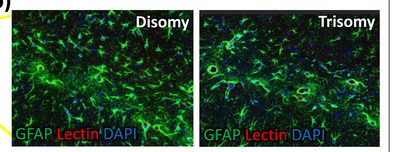
Fig. 4 Altered glutamate homeostasis in DS. a) *Graphic illustration of the functional role of glutamate in the brain.* ***b)*** *Differentially expressed glutamate transport genes.* ***c)*** *Quantification of intracellular glutamate between isogenic pair, as well as a non-DS (female) and two DS (male and female) iPSC-derived astrocyte lines.*

(b) Glutamate transport gene expression (upregulated, downregulated):
SLC17A8 GRIK1 GRIK2 OLFM2 OLFM3 CPT1C GRIA1 GRIA4



Dysfunctional Angiogenesis





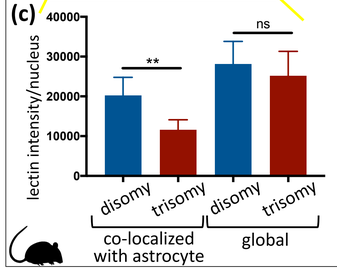


Fig. 5 Dysfunctional angiogenesis in the DS mouse model. a) *Mouse brain slice depicting locations of representative images and lectin intensity analysis.* ***b)*** *GFAP (green), lectin (red) and DAPI (blue) staining in the hippocampi of WT and Ts65Dn mouse model brains.* ***c)*** *Analysis of lectin intensity per nucleus in the mouse hippocampus, co-localized with GFAP and globally. n=3x2 (WT), n=4x2 (DS)*

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