Transcriptional profiling revealed impairment of early brain development in hiPSC-derived models of Alexander disease

Zuzana Matusova[1,2], Werner Dykstra[3], P. Abaffy[1], Elly M. Hol[3], Milos Pekny[4,5,6], Yolanda de Pablo[4], Henrik Ahlenius[7], Oskar Zetterdahl[7,8], Lukas Valihrach[1,9]

[1] Laboratory of Gene Expression, Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czechia, [2] Faculty of Science, Charles University, Prague, Czechia, [3] Department of Translational Neuroscience, University Medical Center Utrecht Brain Center, Utrecht, The Netherlands, [4] Laboratory of Astrocyte Biology and CNS Regeneration, Center for Brain Repair, Department of Clinical Neuroscience, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden, [5] Florey Institute of Neuroscience and Mental Health, Parkville, VIC, Australia, [6] University of Newcastle, Newcastle, NSW, Australia, [7] Stem cells, aging and neurodegeneration lab, Lund Stem Cell Center, Lund, Sweden, [8] Glial and neuronal biology lab, Lund Stem Cell Center, Lund, Sweden [9] Department of Cellular Neurophysiology, Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czechia

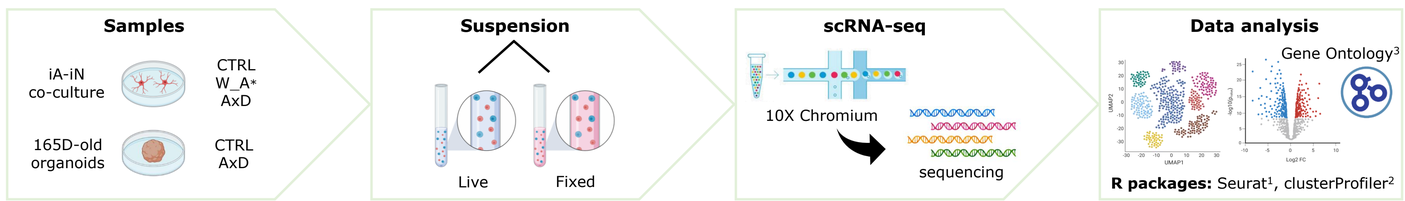
Contact: zuzana.matusova@ibt.cas.cz, lukas.valihrach@ibt.cas.cz

Introduction

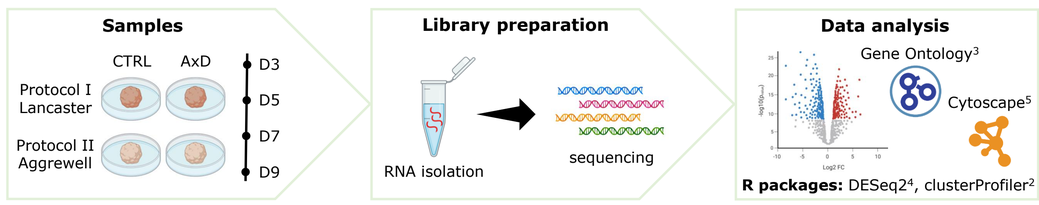
Alexander disease (AxD) is a neurodegenerative disorder caused by mutations in astrocyte intermediate filament protein GFAP. Effects of this mutation can be studied on the human genetic background using models derived from human induced pluripotent stem cells (hiPSCs). Furthermore, such models allow for studying developmental aspect of the GFAP mutations, which has not been addressed before. Here, we used RNA sequencing methods to investigate cell type composition and transcriptional changes resulting from a single point GFAP mutation in 2D co-cultures of neurons and astrocytes and in brain organoids.

Methods

Single-cell RNA sequencing



Bulk RNA sequencing



Results

1) Co-cultures of astrocytes and neurons

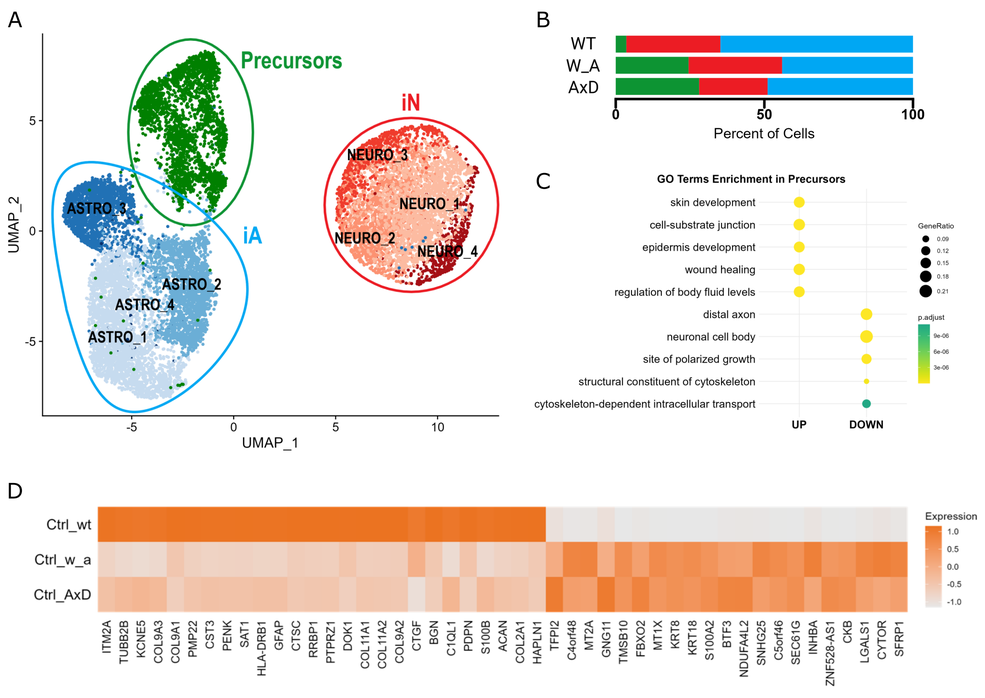


Figure 1: Populations of induced astrocytes, neurons, and precursors were identified in the dataset **(A)**. The precursor cluster was more abundant in GFAP mutation-containing co-cultures **(B)**. This cluster contained cells induced to become astrocytes, which did not fully differentiate. They upregulated adhesion and cytoskeletal molecules, and downregulated neural tissue development **(C)**. DEA of the iA cluster showed downregulation of astrocyte markers (*GFAP*, *S100B*) suggesting their lower differentation status in W_A and AxD co-cultures, and upregulation of metallothioneins implicating an increased level of stress **(C)**.

2) 165-D old cerebral organoids

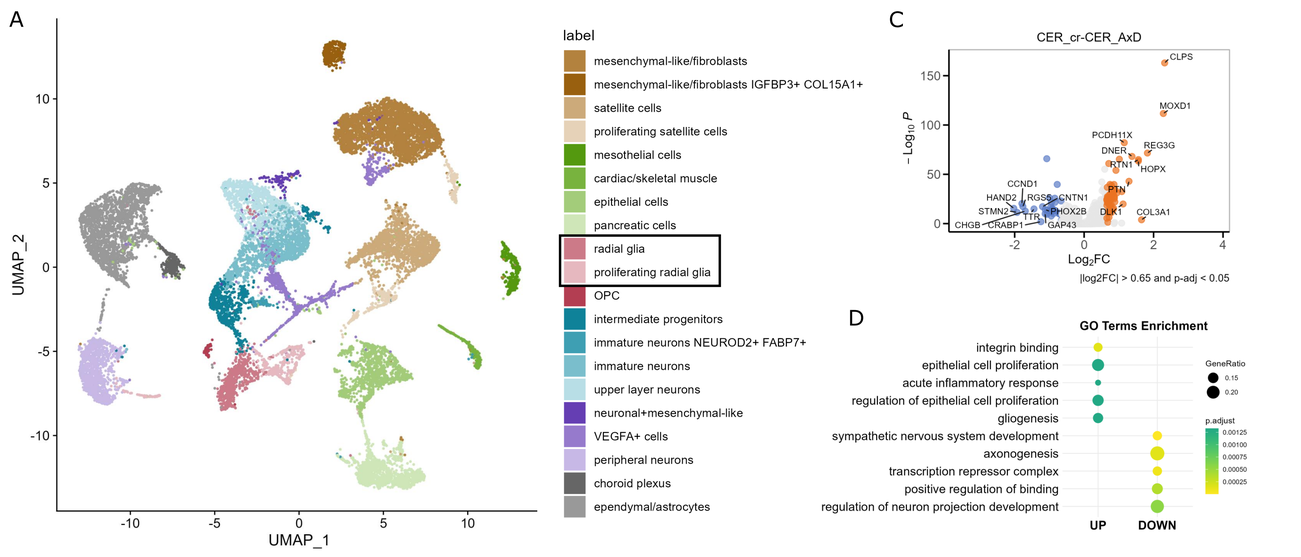


Figure 2: Cerebral organoids contained a variety of cell types, which were differentially represented between conditions. While astrocytes were found almost exclusively in controls, pancreatic-like and mesoderm-derived cells were enriched in AxD **(A, B)**. DEA of radial glia showed downregulation of neuronal markers and upregulation of outer radial glia and pancreatic-like markers **(C)**. Results from GO enrichment analysis suggested dysregulation of membrane components and neural tissue development **(D)**.

3) Early cerebral organoids

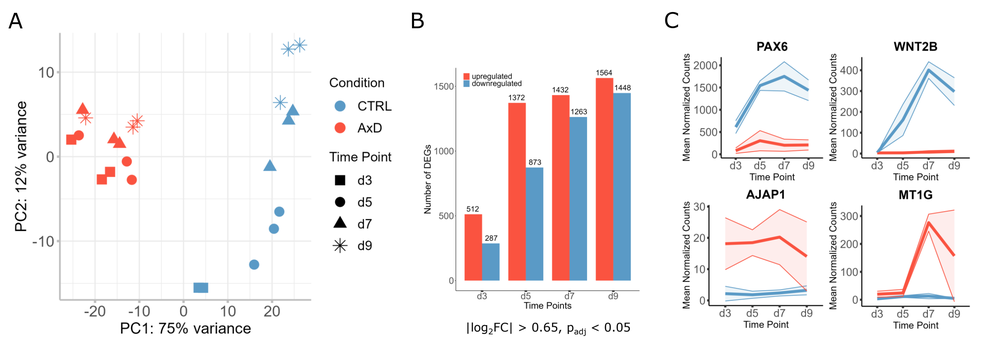


Figure 3: Using the Lancaster protocol, embryoid bodies were grown in separate wells for the first few days of cultivation. PCA showed a significant delay in differentiation of the AxD samples **(A)**. DEGs were identified already at day 3 and their number increased in time **(B)**. The identified changes included downregulation of neuroectodermal markers, dysregulation of major developmental pathways, membrane-related molecules, and increased stress in AxD embryoid bodies across time points **(C)**.

4) Stressed early cerebral organoids

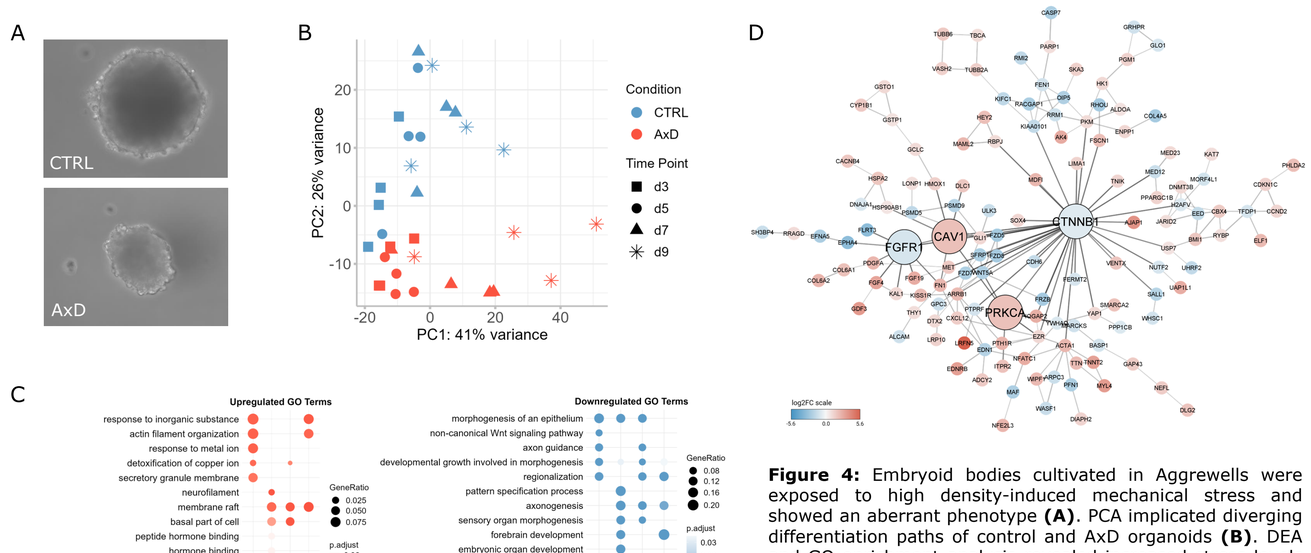


Figure 4: Embryoid bodies cultivated in Aggrewells were exposed to high density-induced mechanical stress and showed an aberrant phenotype **(A)**. PCA implicated diverging differentiation paths of control and AxD organoids **(B)**. DEA and GO enrichment analysis revealed increased stress levels at day 3, upregulation of membrane, cytoskeleton, and ECM components, and markers of mesoderm-derived cell types. Neural tissue development was downregulated **(C)**. Protein-protein interaction network of day 3 DEGs showed four hub genes including β-catenin, directly linked with actin, mechanosensing, adhesion, and WNT related genes **(D)**.

Abbreviations: iA – induced astrocytes, iN – induced neurons, GO – Gene Ontology, DEA – differential expression analysís, ECM – extracellular matrix, OPC – oligodendrocyte progenitor cells, PCA – principal component analysis, DEGs – differentially expressed genes

Conclusions

- all models consistently showed differentiation impairment
- astrocytes did not fully differentiate
- the changes were apparent already at embryoid body stage
- mechanical stress in GFAP mutant samples affected major developmental signaling and lineage commitment
- GFAP mutation altered mechanical properties of differentiating cells

See more

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References and funding

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