Transcriptional profiling of human cerebral organoids harbouring an Alexander disease-causing GFAP mutation

Z. Benešová [1,3], P. Abaffy [1], W. Dykstra [2], E. Hol [2], ALEXANDER Consortium, L. Valihrach [1]

[1] Laboratory of Gene Expression, Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic
[2] Department of Translational Neuroscience, University Medical Center Utrecht Brain Center, Utrecht, The Netherlands
[3] Faculty of Science, Charles University, Prague, Czech Republic
Contact: zuzana.benesova@ibt.cas.cz

INTRODUCTION

Alexander disease (AxD) belongs to rare severe neurodegenerative disorders. It is caused by mutations in an intermediate filament protein GFAP, an important component of cytoskeleton expressed primarily by astrocytes[1]. Effects of these mutations can be effectively studied using human cerebral organoids differentiated from patient-derived induced pluripotent stem cells (iPSCs), as they allow modeling diseases' phenotype on the human genetic background[2].
AxD patient-derived brain organoids exhibited an aberrant phenotype in comparison with their isogenic controls already in early stages of development. In this study, we performed bulk transcriptomic analysis on these young organoids in order to identify changes of gene expression preceding and accompanying the observed developmental impairment. To better identify genes and pathways potentially linked with the AxD, we compared our data also with a previously published dataset[3].

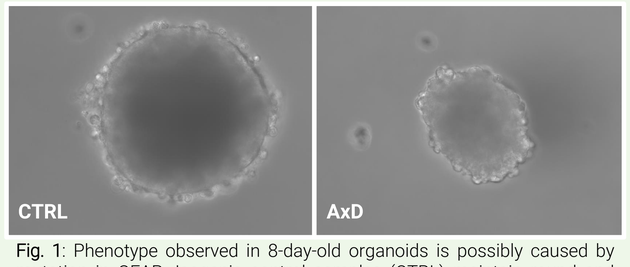


Fig. 1: Phenotype observed in 8-day-old organoids is possibly caused by mutation in GFAP. Isogenic control samples (CTRL) maintain round and regular shape whereas diseased organoids appear small, asymetrical and struggling with development. Organoids above are derived from 825 cell line and captured under 20x magnification.

RESULTS

Differential Expression Analysis

- ✓ GFAP is expressed at a very low level
- ✓ dysregulated genes are present since day 3
- ✓ cell lines share these genes with one another and also with the reference dataset

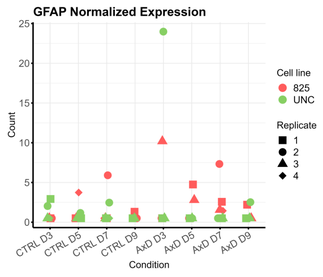


Fig. 2: Very low expression of GFAP was detected in our samples. Nevertheless, a mutation in this protein seems to affect the early development of brain organoids.

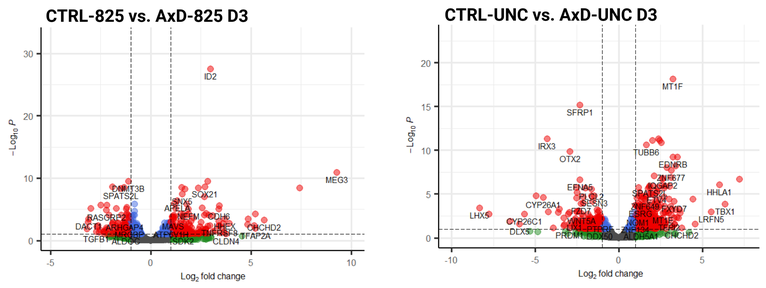
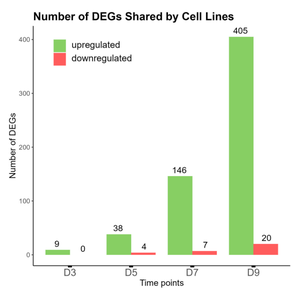


Fig. 3: Volcano plots are showing a high number of differentialy expressed genes (DEGs) in AxD organoids compared to isogenic controls already at day 3 in both studied cell lines (825, UNC). Genes with |log2FC| > 1 and padj < 0.1 were considered significantly up- or downregulated and are highlighted by red colour.



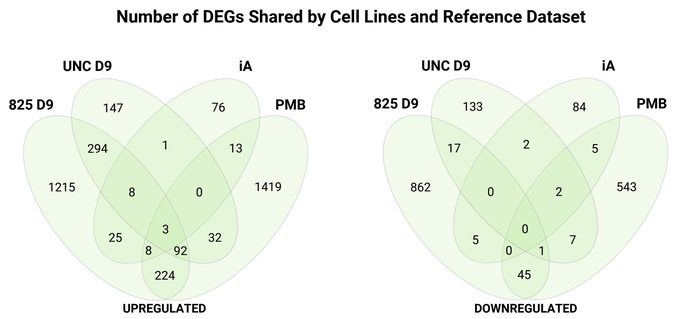
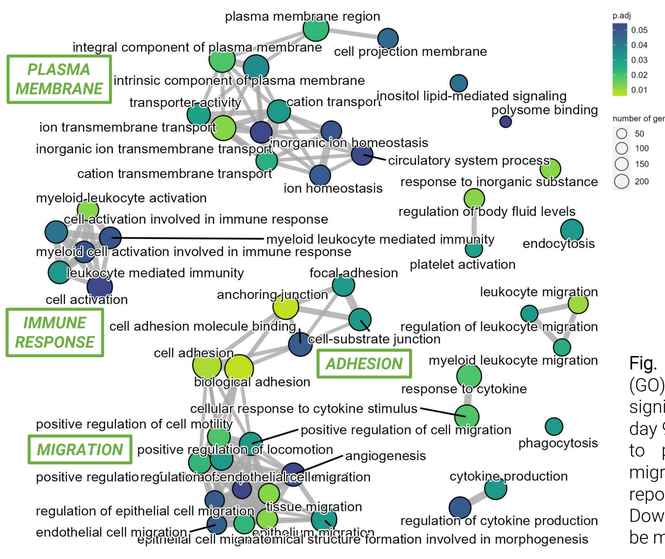


Fig. 4: AxD cell lines 825 and UNC share multiple genes in each time point. The amount of common upregulated genes steeply increases in time. Downregulated genes follow a similar trend, although at lower numbers. Several upregulated genes in both cell lines were also found in the dataset published by Li *et al.* 2018[3], where patient-derived induced astrocytes (iA) and *post mortem* brain samples (PMB) were investigated. At day 9, three genes are present in all four compared gene sets. Fewer common downregulated genes were identified and none of them is shared across the four gene sets.

Gene Set Enrichment Analysis (GSEA) Using Gene Ontology Database

- ✓ terms related to plasma membrane, adhesion, migration and immune response are upregulated in AxD samples
- ✓ terms linked to mitochondrial and ribosomal functions are downregulated in 825 cell line

UNC D9 Upregulated Terms



825 D9 Downregulated Terms

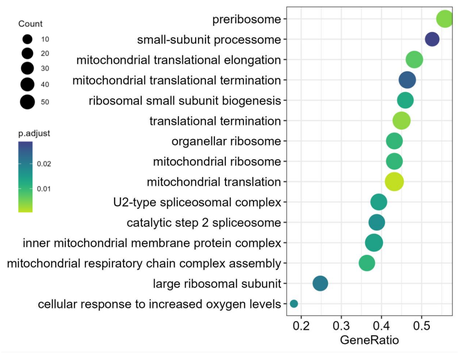
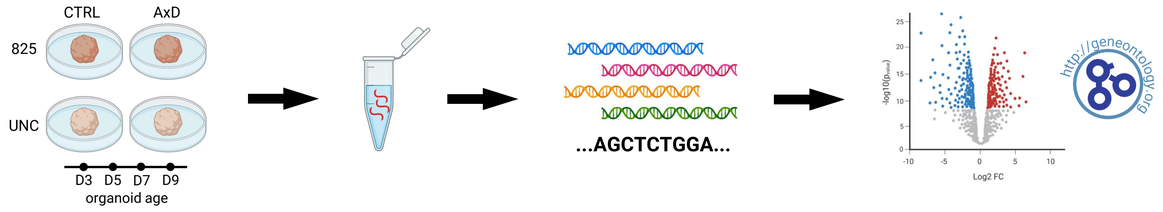


Fig. 5: GSEA revealed a high number of dysregulated Gene Ontology (GO)[4,5] terms in our samples. Padj < 0.1 was considered as the significance threshold and only top 50 (15 resp.) terms are visualized. At day 9 the upregulated terms in UNC line shown here were mostly related to plasma membrane, transmembrane transport, cell adhesion, migration, but also immune response activation. These terms were also reported previously in iA cultures and *post mortem* brain samples[3]. Downregulated GO terms in the 825 line, where the phenotype seems to be more pronounced, include mitochondrial and ribosomal processes.

METHODS



SAMPLE PREPARATION — **RNA ISOLATION** — **LIBRARY PREPARATION AND SEQUENCING** — **DIFFERENTIAL EXPRESSION AND ENRICHMENT ANALYSES**

SAMPLE PREPARATION

- organoids differentiated from 2 patient-derived iPSC lines (AxD-825, AxD-UNC)
- organoids differentiated from isogenic control iPSC lines with corrected mutation (CTRL-825, CTRL-UNC)
- samples harvested at 4 timepoints (day 3, 5, 7 and 9)

LIBRARY PREPARATION AND SEQUENCING

- sequencing library prepared using QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina
- samples sequenced on Illumina NovaSeq Platform

DIFFERENTIAL EXPRESSION AND ENRICHMENT ANALYSES

- sequencing data preprocessed using UMI-tools, Trimmomatic, SortMeRNA and STAR
- data analyzed using R packages DESeq2[6] (differential expression) and clusterProfiler[7,8] (GSEA)

META ANALYSIS

CONCLUSIONS

- The two studied iPSC lines differ in transcriptomic changes and the timepoint of their onset during the early organoid development.
- Very low expression of GFAP was detected at the mRNA level. However, the effect of its mutation appears to be substantial. Follow-up experiments will attempt to further explain this phenomenon.
- A number of genes differentially expressed between CTRL and AxD was detected already at day 3 of organoid development.
- Gene Ontology terms related to cell adhesion, migration and development are dysregulated and are to some extent shared by both cell lines.
- Mutation in GFAP might also result in dysregulation of mitochondrial and ribosomal functions, as they naturally interact with cytoskeleton.
- Common DEGs and GO terms were found in comparison with a published dataset[3].
- This experiment is currently validated by RT-qPCR and immunohistochemistry and provides a baseline for follow-up experiments including single-cell RNA sequencing.

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Support:
The project was supported by Ministry of Education, Youth and Sports, under the frame of EJP RD, the European Joint Programme on Rare Diseases: CZ.1.05/1.1.00/02.0109 and RVO 86652036. In addition, this project has received funding from the European Union's Horizon 2020 research and innovation programme under the EJP RD COFUND-EJP N° 825575.