Expression of cellular and neurodevelopmental markers in cerebral organoids

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OVERVIEW

Aim:
To characterize cellular protein markers in brain organoids during neurodevelopment and maturation

Methods:
Ultra-high Performance LC, tandem mass spectrometry

Achievements:
Developed protein assays for cell-type specific markers

INTRODUCTION

- **The dementia** epidemic affects 47 million people globally, with **increasing incidence**. [1]
- **Cerebral organoids** - an emerging model system **to study** neurodegenerative diseases. [2]
- Requirement of prior **characterization** in terms of cellular composition and maturation of the organoids. [3, 4]
- **Protein markers of specific cell types** and **neuronal aging** are quantifiable using selected reaction monitoring assays.
- Tissue levels of cellular and neurodevelopmental markers attributable to a **specific cell type** were determined in cerebral organoids.

METHODS

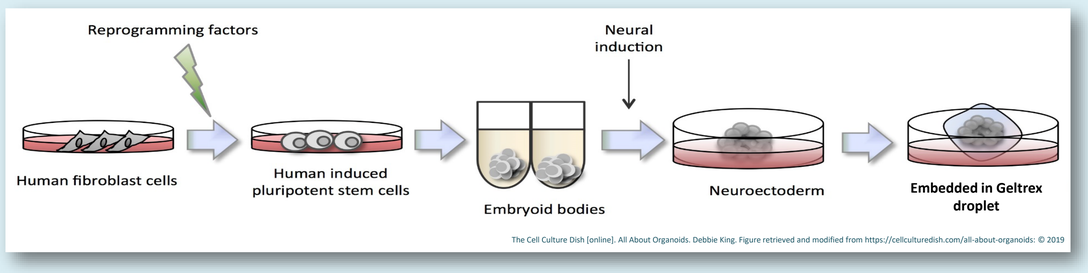

Fig. 1: Cerebral organoids cell culture protocol by Lancaster et al. [5]

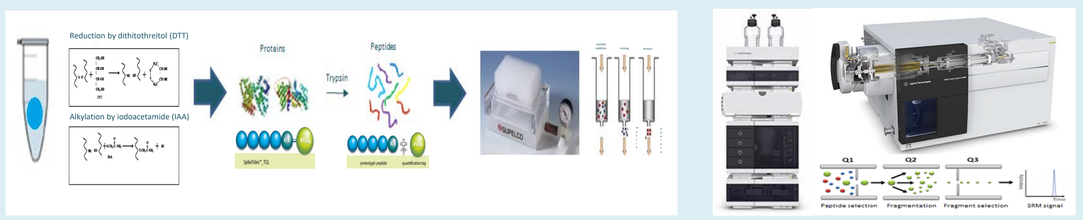

Fig. 2: Workflow of quantitative protein assays by selected reaction monitoring (SRM) tandem mass spectrometry **a) Protein isolation,** Reduction and Alkylation **b)** Enzymatic protein **digestion (trypsin) c) S**olid **P**hase **E**xtraction **d) UHPLC** (C18 Peptide CSH column)**-tandem mass spectrometer** (6495B series, Agilent technologies, CA, USA) used for investigation of cell markers in positive mode.

Fig. 4: Cellular and neurodevelopmental markers determined in organoids.
Immature neurons differentiated from progenitor cell produced by asymmetrically dividing radial glia: **DCX**, **TUJ**; radial glia transforming from NE cells during neurogenesis: **BLBP**; terminally differentiated mature neurons: **MAP2** and **GRIN1.**

RESULTS

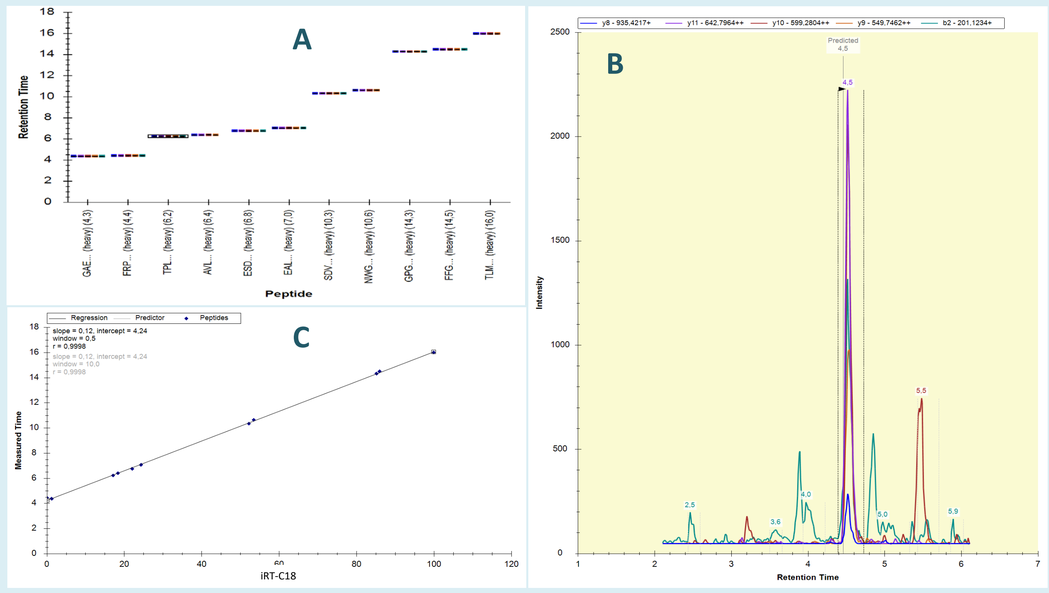

Fig. 5: Workflow for **identification** of proteotypic peptides of cellular protein markers using isotopically labeled **standards** as retention indices for a **prediction model**. (A) The iRT Calculator was recalibrated using retention indices covering the chromatography gradient. (B) Linear regression of the standard peptides iRT values. (C) Skyline software for peptide identification based on predicted retention time and product ions.

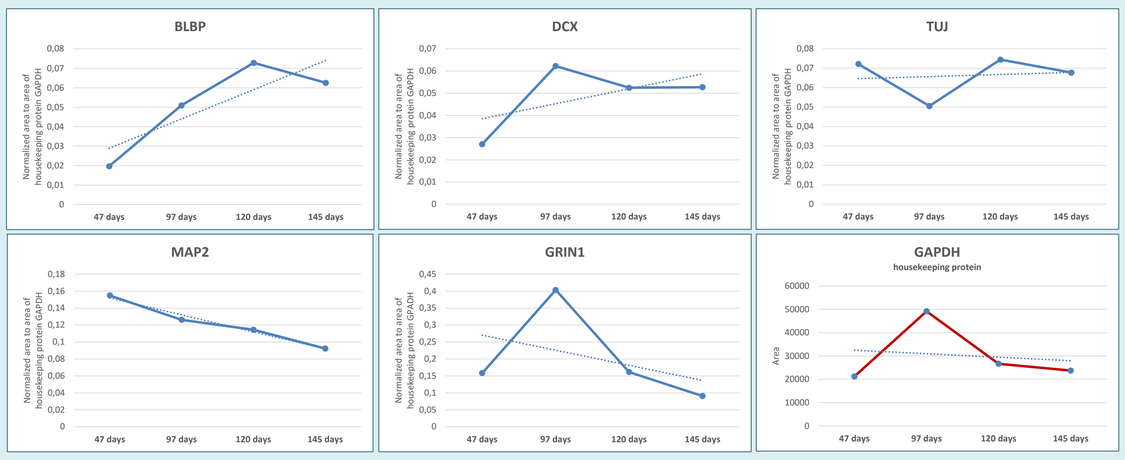

Fig. 6: Levels of cell markers at different developmental stages of brain organoids. Neuronal markers of neurogenesis (e.i. **DCX** and **TUJ**) show stable levels in matured organoids. **Increasing** tissue levels of **BLBP** indicate ongoing neuronal differentiation. **Decrease** in number of neuronal cells corresponds to decrease in **MAP2** and **GRIN1** levels in organoid.
Level of each protein marker was normalized to housekeeping protein **GAPDH**, widely expressed across cell populations and thus corresponding to the total cell count.

CONCLUSIONS

- Multiplex SRM assay to **determine cellular markers** of **neurodevelopment** and **maturation** in organoids.
- **Changes in cellular markers tissue levels were explored in aging organoids (47, 97, 120, 145 day of cell culture)**.

References
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