Characterization of aging cerebral organoids – an emerging model system to study Alzheimer´s disease

Markéta Nezvedová [1], Tereza Váňová [2,3], Dáša Bohačiaková [2], **Zdeněk Spáčil** [1]

(1) Masaryk University, Faculty of Science, RECETOX Centre, Brno, Czech Republic
(2) Masaryk University, Faculty of Medicine, Dept. of Histology and Embryology, Brno, Czech Republic
(3) International Clinical Research Center (ICRC) St. Anne's University Hospital, Brno, Czech Republic
marketa.nezevdova@recetox.muni.cz

OVERVIEW

Aim: Characterization of cerebral organoids during neurodevelopment and maturation by identification of cellular protein markers.
Methods: Ultra-high Performance LC, tandem mass spectrometry (UHPLC-SRM-MS/MS)
Achievements: Development of multiplex protein assay for cell-type specific markers.

BACKGROUND

- **The dementia** epidemic affects 47 million people globally, with **increasing incidence**.[1]
- Previous research mainly focused on the aggregation of β-Amyloid and Tau protein.
- Recent observation point to neuroinflammation as potential cause of Alzheimer´s disease (AD).
- **Cerebral organoids** - an emerging 3D model system **to study** neurodegenerative diseases with the advantage of accelerated temporal maturation (4-5 months).[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.

EXPERIMENTAL DESIGN AND METHODS

Fig. 1: Cultivation of **cerebral organoids** following the protocol published by Lancaster et al.[5] Organoids derived from fibroblasts of an **AD** patient and a **healthy** control were collected at six time points.

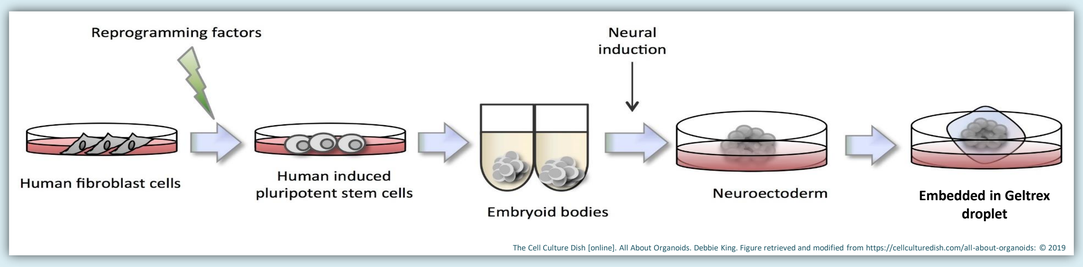


Fig. 2: Cellular and **neurodevelopmental markers** determined in organoids.

- **Neuroepithelial (NE) cells -** symmetrically dividing cells expressing SOX2, the earliest marker of the neural plate, essential for maintaining self-renewal. It is expressed in proliferating cells and those acquiring glial fates, downregulated in post-mitotic neurons. During neurogenesis, NE cells transform into **radial glia cells**. Glial hallmarks (BLBP) begin to emerge, including **astrocyte** (star-shaped glial cells) markers – S100beta, GFAP. Radial glia divide asymmetrically to produce one radial glia cell and one intermediate progenitor cell (IPC). IPCs differentiate into post-mitotic **immature neurons** (TUJ, DCX promoting neurite extension and cell migration) migrating to their final destination in the nervous system and integrate into the neuronal network. **Mature neurons** - terminally differentiated, no longer able to divide.
- To develop a multiplex protein assay based on SRM-MS/MS - Human protein database **neXtprot** (online) was used for the selection of proteotypic peptides, subsequently **SRMAtlas** (online) to create an instrument method (SRM transitions, collision energies) for MassHunter (an acquisition software for the QqQ mass spectrometer.

Gene name	Protein name	Expressed in
SOX2	SRY-Box Transcription Factor 2	**Neuroepithelial cells**
BLBP	Fatty acid-binding protein, brain	**Radial glia**
DCX	Neuronal migration protein doublecortin	**Immature neurons**
TUJ	Tubulin beta-3 chain	**Immature neurons**
MAP2	Microtubule-associated protein 2	**Mature neurons**
NF medium	Neurofilament Medium	**Mature neurons**
SYN1	Synapsin-1	**Mature neurons-synapses**
S100beta	Calcium binding protein	**Astrocytes**
GFAP	Glial fibrillary acidic protein (GFAP)	**Astrocytes**

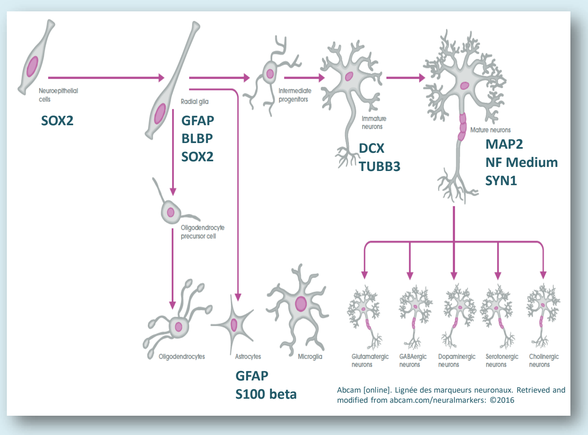
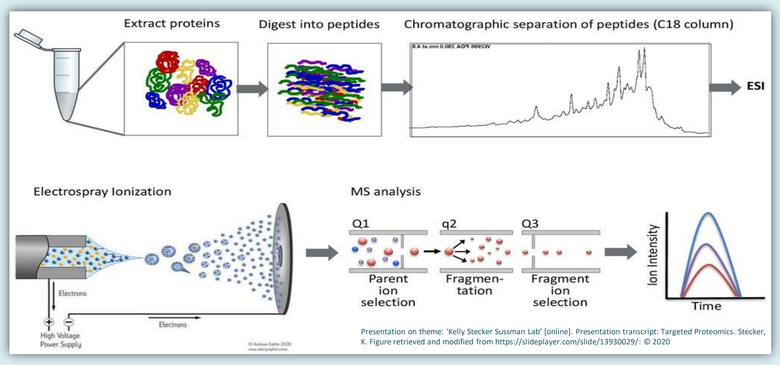
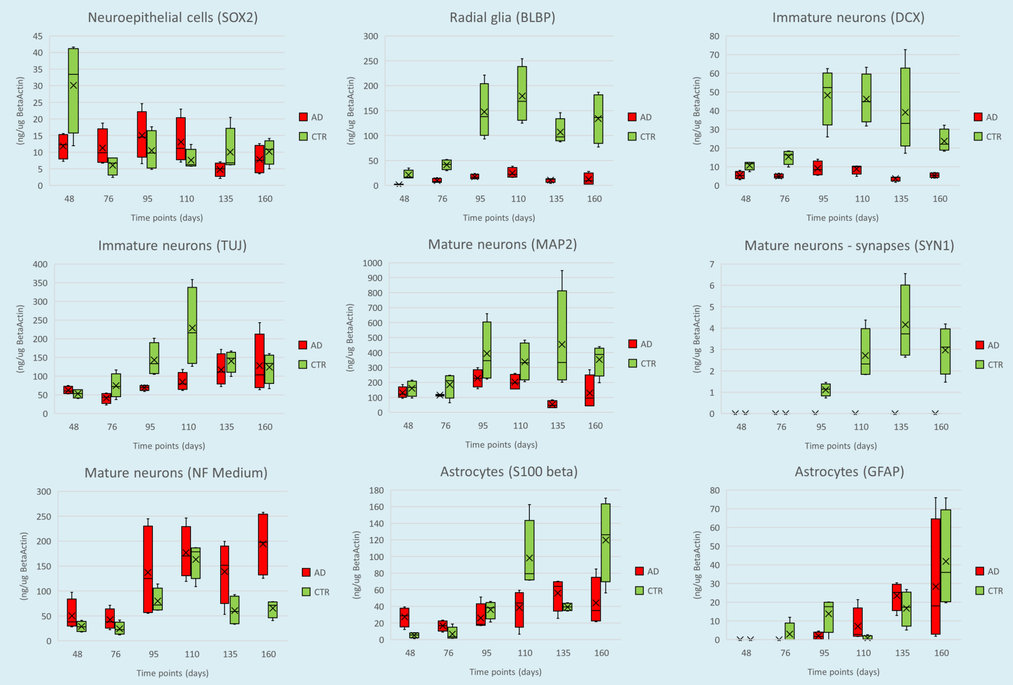


Fig. 3: Bottom-up proteomics protocol consisted of the enzymatic digestion by trypsin to obtain peptides for investigation of cell markers. **UHPLC** (C18 Peptide CSH column)**-tandem mass spectrometer** (6495B series, Agilent technologies, CA, USA) used for investigation of cell markers in positive mode.



RESULTS

Fig. 5: Levels of cell markers identified in cerebral organoids (CO, n=4) derived from AD vs. control (CTR) cell lines at various developmental stages of cerebral organoids.
Neuronal marker of neurogenesis (**SOX2**) showed stable levels in both, AD and CTR organoids. **Increasing** tissue levels of **BLBP** indicate ongoing neuronal differentiation with culmination in 110 days for both, AD and CTR CO. **DCX** is microtubule-associated protein in dendrites and shows **decreasing** trend in older AD CO. **Decrease** in **MAP2** levels in AD CO corresponds to relative decrease in number of mature neuronal cells caused by **generation** of **new cell population** in organoid (**TUJ**) **–** possible explanation for AD organoids. Marker for synapses (**SYN1**) was detectable only in CTR CO appearing in 95 days old organoids. Both markers for astrocytes showed **increasing** trend in AD and CTR cell lines. Generally all identified protein markers were detected in higher concentration in CTR organoids, except for **NF Medium**.
Level of each protein marker was normalized to housekeeping protein **BetaActin**, widely expressed across cell populations and thus corresponding to the total cell count.



Future plans:

- To optimize the **tissue proteomics** protocol for protein extraction and sample processing.
- To characterize the cell composition during maturation, neurodevelopment and aging of cerebral organoids derived from **AD** and **healthy age-matched individuals**.
- To apply the in parallel developed multiplex SRM assay for selected potentially new AD biomarkers (a target list of early-onset **neuroinflammatory biomarkers** already generated) to second cultured timeline experiment for proper differential analysis.
- To investigate the **biological role** of newly discovered potential protein biomarkers and **possible cause** of observed changed protein expression.

CONCLUSIONS

- Developed multiplex SRM assay to **determine cellular markers** of **neurodevelopment** and **maturation** in organoids.
- Tissue levels of cellular and neurodevelopmental markers of a specific **cell type** were determined in cerebral organoids.
- **Tissue levels of cellular markers were explored in aging organoids (48, 76, 95, 110, 135, 160 day of cell culture)**
- **Different protein levels were observed between AD and CTR organoids.**

References
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