Mass spectrometry-based protein analysis in research of Alzheimer's disease

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OVERVIEW

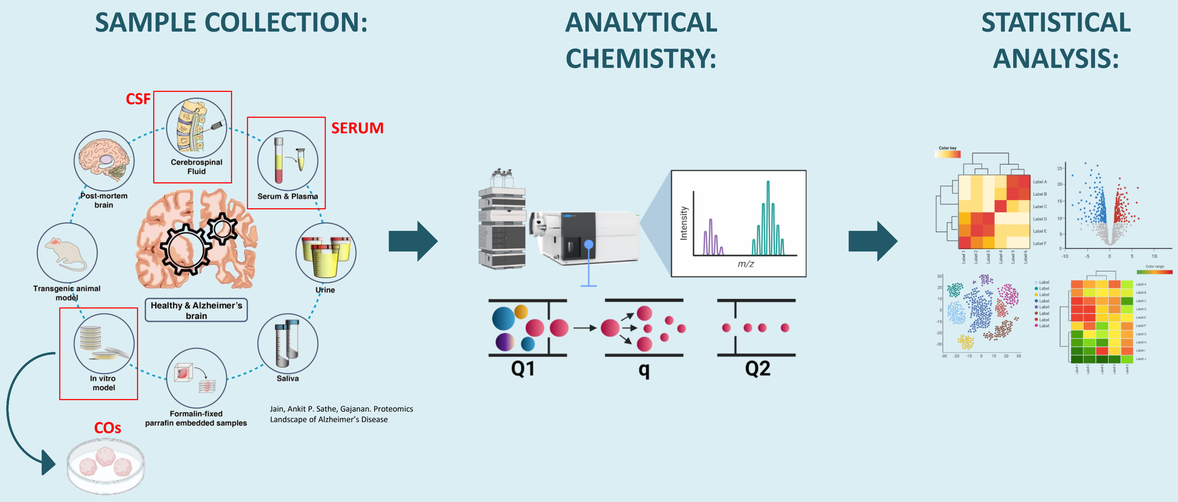
Aim: Identification of cell protein markers to characterize the 3D cell model system of the brain used to study Alzheimer's disease (AD). Quantify a new set of protein biomarkers related to AD in cell systems and clinical samples.
Methods: Ultra-high performance LC, tandem mass spectrometry (UHPLC-SRM-MS/MS)
Achievements: Development of multiplex assay targeting several proteins of interest: cell-specific and AD-related proteins.

BACKGROUND

- **The dementia** epidemic affects around 47 million people worldwide, with **increasing incidence**. Alzheimer's disease (AD) is the most common form of dementia with an unknown cause. [1]
- Recent advances in **mass spectrometry** and cell biology opened new avenues to investigate neurodegeneration. [2,3]
- **Cerebral organoids** (COs) are three-dimensional cell cultures representing an emerging model system to study biological processes causing neurological diseases.
- COs recapitulate the brain tissue's cytoarchitecture, mimicking the brain development in vivo.
- Immune-based assays are frequently used to analyze proteins.[4]
- **Several protein targets** can be analyzed **simultaneously** using the system of ultra-high performance liquid chromatography (**UHPLC**) coupled to tandem mass spectrometry in the mode of selected reaction monitoring (**SRM-MS/MS**).

EXPERIMENTAL DESIGN AND METHODS

Fig. 1: Formulation of **working hypothesis using cerebral organoids** and **confirmation** of working hypothesis **in clinical samples (cerebrospinal fluid, serum).**

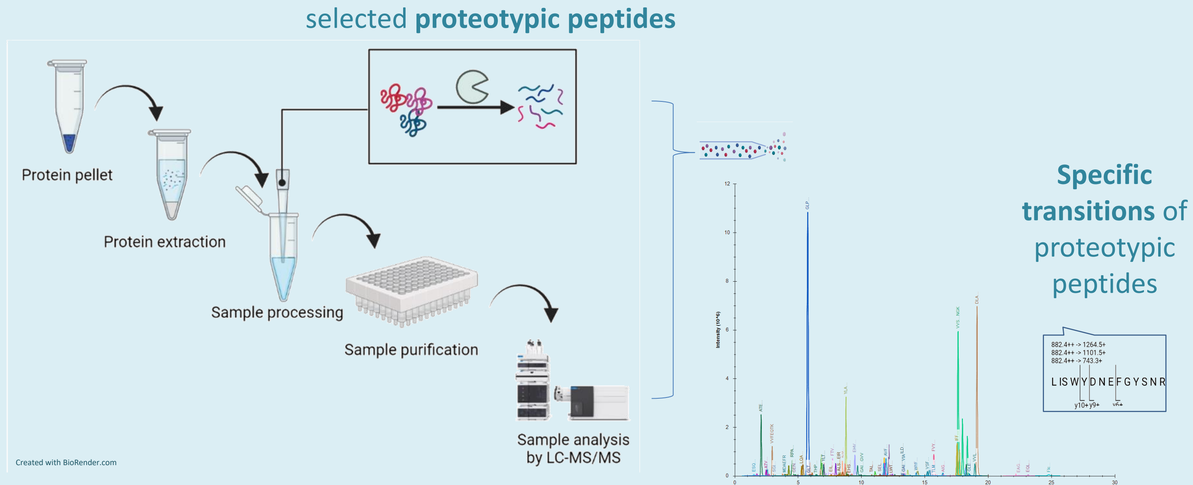


Sample types used to study proccesses occuring in the brain **are highlighted**.

UHPLC (C18 Peptide CSH column) coupled to **tandem mass spectrometer** (Agilent) was used for targeted analysis.

Use of appropriate **bioinformatics** tools to demonstrate **significance** in the obtained data sets.

Fig. 2: Bottom-up proteomics. The sample preparation protocol consisted of the enzymatic digestion by trypsin to obtain peptides to investigate protein cell markers. **SRM-MS/MS assay** development to **analyze peptides unique for** targeted **proteins**.



RESULTS

CEREBRAL ORGANOIDS

Fig. 3: Characterization of cerebral organoids (COs, n=4 per time point) using cell-specific protein markers. (A) Cell markers identified in COs derived from healthy (wild type, WT) cell lines at various developmental stages showed the usual course of neurodevelopment. **Decreasing** tissue levels of NSCs markers and **increasing** RGCs markers indicate ongoing neuronal differentiation with a culmination in D110, same as neuronal markers. Astrocyte markers showed an **increasing** trend with the highest levels in the last time point. **(B)** Cell markers indicate normal neurodevelopment in both, WT and diseased (mutation in PSEN1, MUT) organoids. **(C)** Successful identification of 27 of 40 selected protein markers in each analyzed cerebral organoid, including the smallest one (6 µg).

***NSCs**=Neural stem cells, SOX2;*
***RGCs**=Radial glial cells, FABP7; **Neurons**=TUBB3; **Astrocytes**=CD44*

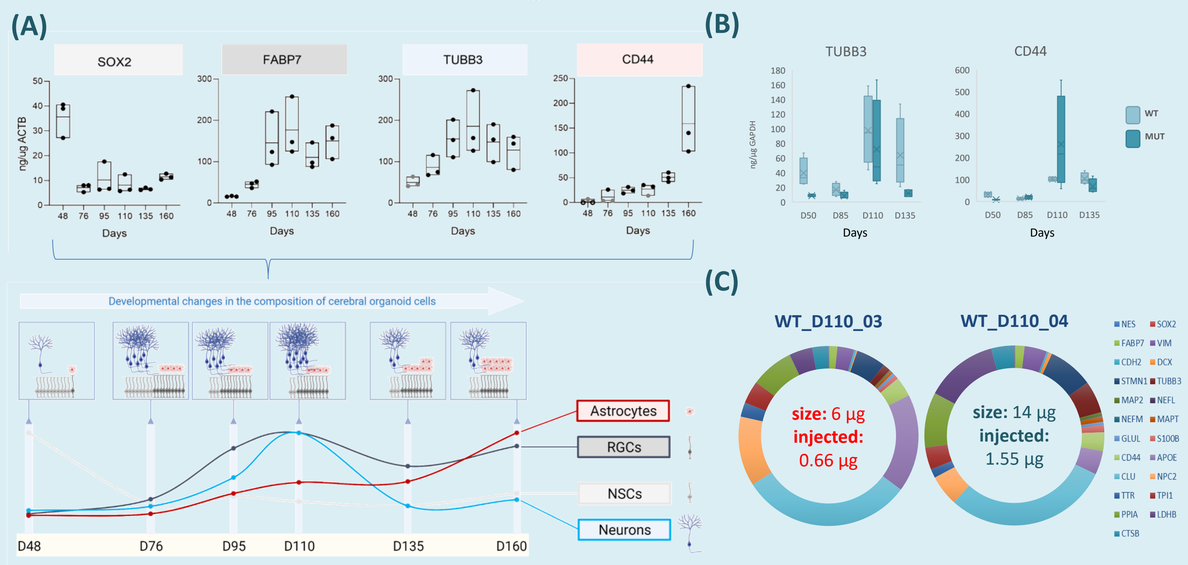
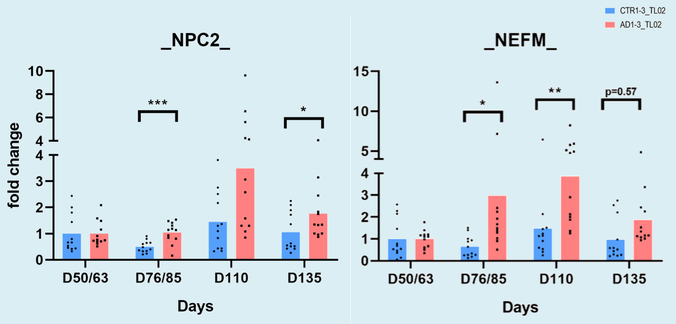


Fig. 4: AD-related proteins in three different cell lines from healthy controls (CTR1-3, n=12) and patients with Alzheimer´s disease (AD1-3, n=12). Levels of markers related mainly to brain lipids (**NPC2**), and neurofilaments (**NEFM**) showed increased levels in AD organoids.



CLINICAL SAMPLES

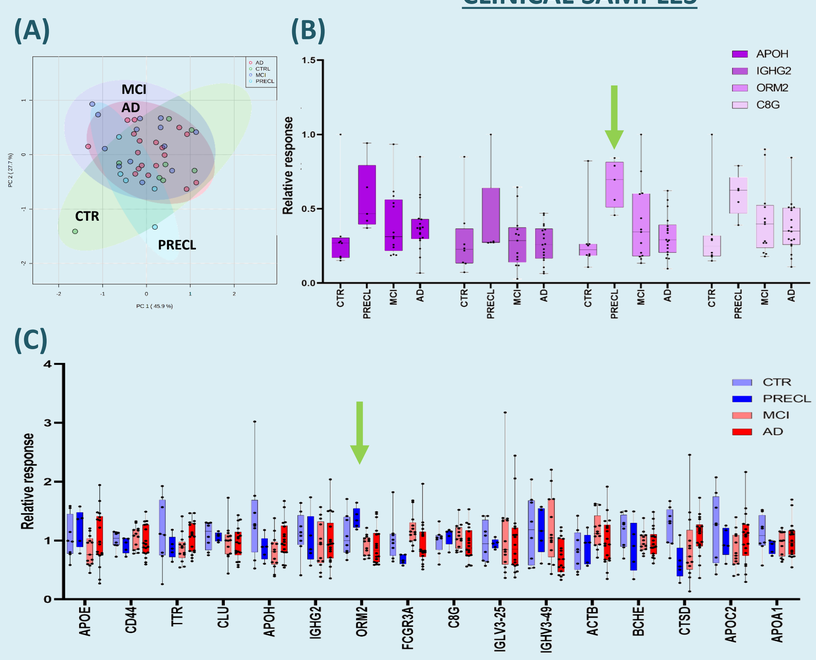


Fig. 5: Analysis of CSF (A, B) **and serum** (C) **samples** derived from healthy control (CTRL, n=8), preclinical stage (PRECL, n=5), mild cognitive impairment (MCI, 13) and patients with AD (n=18).
(A) PCA analysis did not show separated groups for CSF samples. **(B)** Selected proteins (n=4 **out of 29**) showing increased levels **in PRECL CSF samples**.
(C) Proteins (**n= 13**) determined in serum samples with highlighted **ORM2 showing a similar trend as in CSF**.

SUMMARY *AND PLANS*

- **The COs** were found **as *in vitro* model systems** that **recapitulate *in vivo* developmental features** (same in CTR vs. diseased COs) and thus suitable for **studying biological processes** occurring in **human brains during ageing**.
- Analysis of COs brought a set of markers with altered levels of protein markers related mainly to **lipid metabolism and/or amyloid β binding**.
- In CSF and serum samples, another spectrum of protein markers was observed, including proteins related to the **immune system**.
- *The newly discovered potential protein biomarkers found **in COs, CSF, and serum** samples will be further investigated in terms of their role in the pathogenesis of AD.*

References
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