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CESI-MS of neuronal cultures differentiated from ALS patient-derived iPSCs: a model for personalized high throughput perturbation screening

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Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a devastating incurable disease. It involves the destruction of motor neurons and neural connections in the brain and spinal cord, resulting in progressive loss of all voluntary muscle function. Patients gradually become paralyzed, losing their ability to walk, eat and breath unassisted, with death usually occurring around 2–5 years after diagnosis. Analysis of human motor-neurons differentiated from patient-derived iPSCs, as cultured organoids or as an organ-on-a-chip, could provide new insight to the biology of human neurons and a way for patient-specific drug development. The paradigm to move toward personalized therapies for ALS and other diseases, may start by high-throughput, low sample quantity proteomic quantitative screening of iPSC-derived organoids for 100s of individuals. This study is the start of building such a pipeline.

Focusing on the early signals of ALS, motor neuron cell cultures were derived from iPSCs generated from healthy individuals or those with ALS. We used an 18-day differentiation protocol from which no significant transcriptomic difference between ALS and control lines were identified and minimal (less than 25) protein changes were found using a classical nano-DIA-MS workflow on a 6600 Triple TOF. However, when an aliquot from a subset of day 18 iPSC-derived neuron samples were analyzed using a CESI-IDA-MS workflow, both protein abundance and phosphorylated peptides were found to be highly dysregulated. Greater than 1000 proteins represented by >7500 peptides were quantified from approximately 45 ng of protein in a single CESI-IDA-MS run including, ~200 phosphopeptides and >200 acetylated peptides. Higher numbers of quantifiable proteins could be obtained using the CESI-IDA-MS workflow with i) the generation of 2 offline peptide fractionations from the C18 desalting step (~3000 protein identified) or ii) by increasing load from 45 to 60 ng (>1600 protein identifications) for both the cell culture and organ-on-a-chip iPSC-differentiated motor neurons. Microtubule-associated protein 1B (MAP1B), known for its role in neuron development, was the most phosphorylated single protein identified in both the ALS data sets analyzed by CESI. Protein Interaction Network Extractor (PINE), a newly developed user-friendly bioinformatic platform, was used to highlight how many of these sites appear to be dysregulated. Further, based on NetworKIN predictions, several groups of phosphorylated sites on MAP1B are likely co-regulating by the same kinase. The various iPSC-derived neuronal models coupled with proteomics is a feasible way to study the effects of current treatments, test drug efficacy prior to clinical trials and develop new treatments for, not only ALS but all diseases.