Metabolomic profiling of cell lines knockout for Alzheimer's disease related genes

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Background

Alzheimer's disease (AD) is the most common cause of dementia characterized by dysfunction in memory formation systems and cognitive impairment. Accumulating evidence suggests that different molecular entities exhibit synergistic effects in the evolution of the disease, with the dysregulation of interacting proteins being a major cause of the AD pathology.

Objectives

The aim of the present study is to pinpoint the potential effects caused by the malfunction of specific proteins that define AD pathology on neuroblastoma cells and provide complementary information on possible intercellular indicators, causes and pathology of the disease. Through NMR based metabolomic analysis, we investigate the influence of the specific proteins related to AD pathology namely the amyloid precursor protein, presenilin and α-synuclein to whole metabolome.

Methods

Sample preparation

› Four knockout (KO) lines were produced by targeting the genes of the amyloid precursor protein (APPKO) and presenilin 1 protein (PSEN1KO). The α-synuclein gene (SNCAKO) was targeted to differentiate between general and AD-specific neuro-degeneration. The findings were compared to a control line that underwent the same processing but retained all genes intact (KO-).

› Six replicates of cell cultures from each of the four KO lines were collected to obtain statistical results.

› Cell extraction and collection of polar metabolites for the construction of metabolomic profiles.

Metabolomics Data

› High Resolution ^{1}H 1D NMR and *J*-resolved spectra.

› Identification of metabolites: Topspin (Bruker BioSpin), Chenomx Profiler (Chenomx Inc.), Human Metabolome Database (HMDB).

Statistical Analysis

Comparison of metabolite levels across the four different cell lines through appropriate computational programs:

› GraphPad Prism Univariate analysis and

› SIMCA - Multivariate Data Analysis Software.

Results

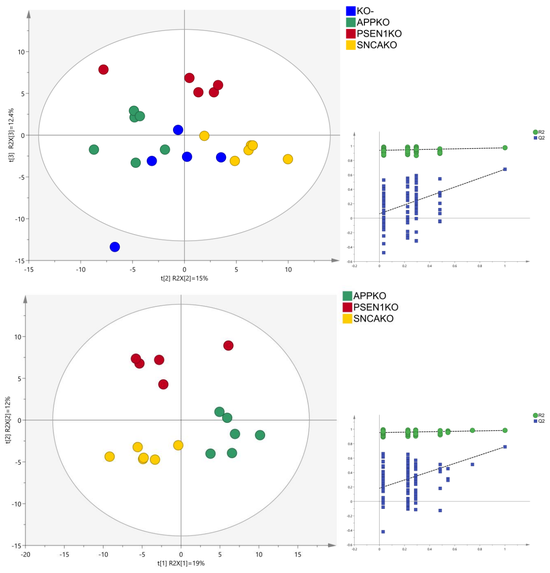

Figure 1: Multivariate analysis (PLS-DA) comparing the metabolome fingerprint of the cell lines, demonstrating distinct profiles, along with the corresponding permutation analysis, showcasing the reliable predictive capability of the model.

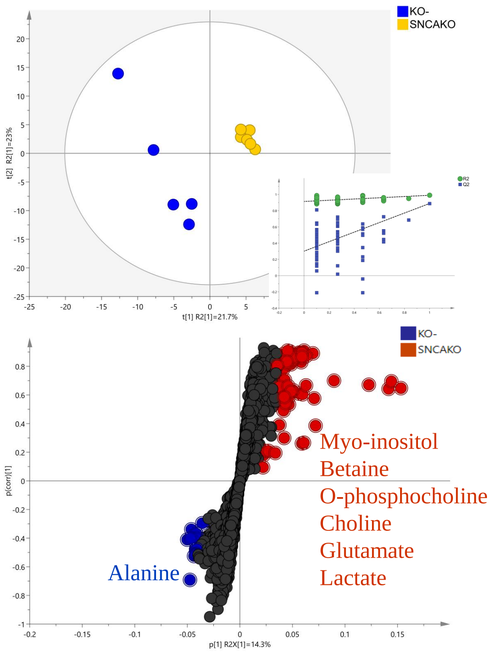

Figure 2: PLS-DA scores plot (above) of the control (KO-) and SNCAKO cell line, showcasing the main metabolites (S-plot) causing the differentiation between the lines.

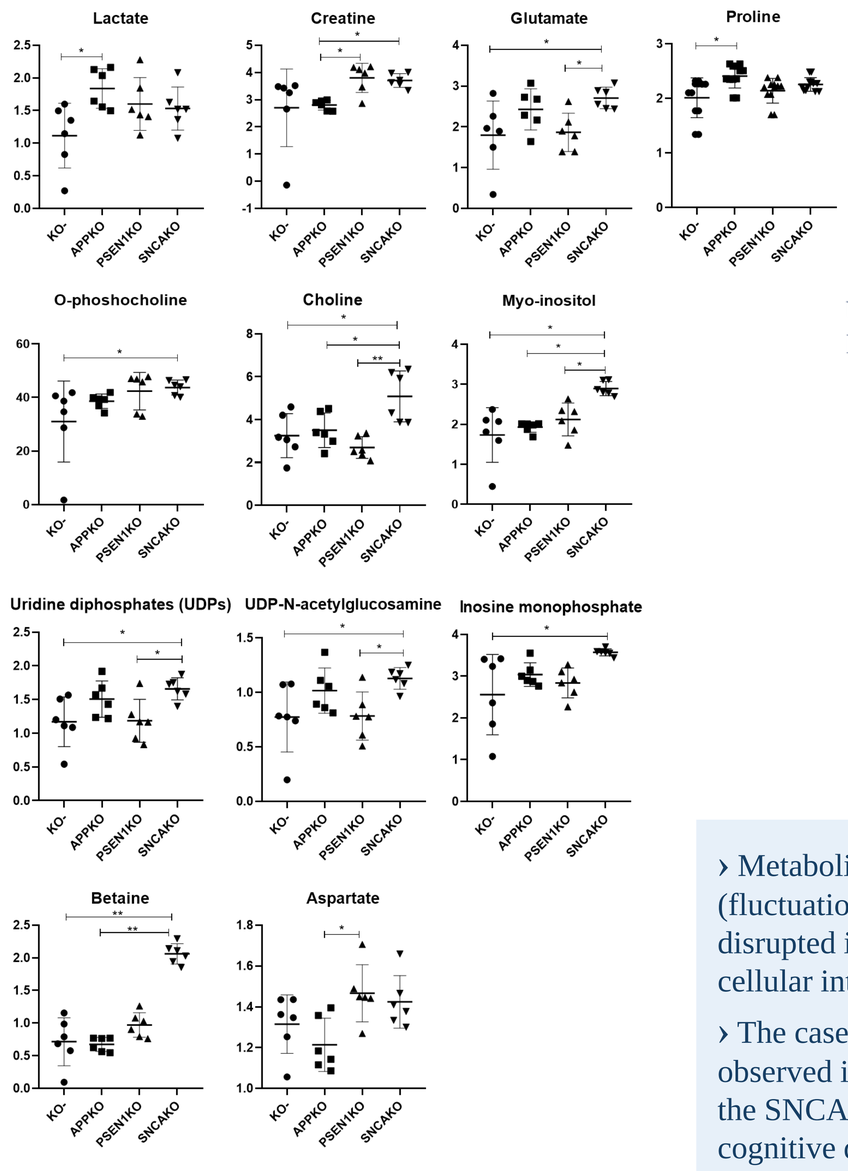

Figure 3: Univariate analysis comparing the different cell lines. Boxplots (mean ±SD) of metabolites with statistically significant difference amongst the modified cell lines.
* signifying p-value <0.05; **<0.001

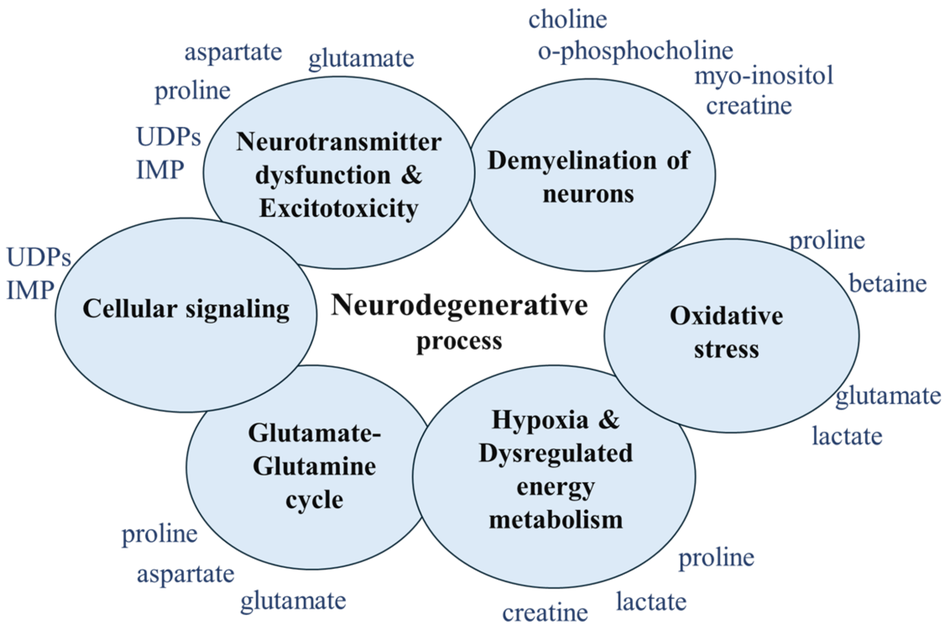

Figure 4: The key metabolic functions of nerve cells involving the metabolites (Fig. 2) that differ significantly in the modified cell lines.

› Metabolic pathways related to phospholipid metabolism in cell membranes (fluctuation of choline, o-phosphocholine, and myo-inositol levels) are primarily disrupted in the SNCAKO cell line, indicating processes of demyelination and loss of cellular integrity in various neurodegenerative diseases.

› The case of nucleotides (UDPs, IMP) is particularly interesting, as an increase is observed in the APPKO and SNCAKO cell lines, with the most significant increase in the SNCAKO line. This calls for further investigation into cellular signaling processes, cognitive dysfunction, and synaptic plasticity.

› Aspartate and glutamate are two of the most important molecules in neurotransmission and normal signaling in a healthy brain. Notably, glutamate exhibits the most significant dysregulation in the SNCAKO cell line, while aspartate is most affected in the PSEN1KO line, revealing a possible connection to Alzheimer's pathology.

› A significant alteration is observed in metabolites involved in neuronal energy homeostasis pathways and redox regulation (lactate, creatine and proline) in the APPKO cell line, highlighting particular interest specifically in the pathogenesis of Alzheimer's disease.

Conclusions

› Metabolome analysis provides a sensitive snapshot of the neuronal cell, thus enabling a detailed examination of any effects resulting from the dysfunction of these specific proteins that define AD pathology and the process of neurodegeneration.

› The results of this study shed more light on the pathology profile as it pertains to malfunctioning intercellular pathways and possible indicators for AD.

› These findings call for future investigations utilizing the valuable tool of metabolomics, perhaps with a larger sample size, spanning different cell ages, for a clearer picture of the progression of the disease.

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