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

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Neurodegeneration comprises a diverse group of progressive disorders of the central and peripheral nervous system. Alzheimer's disease (AD) is the most common cause of dementia characterized by disfunction in memory formation systems and cognitive impairment. Accumulating evidence suggests that different molecular entities exhibits synergistic effects in the evolution of the disease. Specifically, the dysregulation of interacting proteins has been identified as a major cause of the AD pathology. A protein with clear involvement in the progression of the disease is the amyloid precursor protein (APP), with diverse physiological functions in the human adult brain, namely, cell adhesion, intracellular signaling and synaptic and neuronal plasticity. The aggregation of the Aβ peptide plays a central role in the pathogenesis of Alzheimer's disease (AD). Aβ is formed after sequential cleavage of APP, which produces a C-terminal fragment, that is then cleaved through its transmembrane domain by the presenilin complex, generating a series of fragments that include Aβ peptides (released into the lumen) and amyloid intracellular domain [1]. These two interacting proteins are principal in the initiation of the AD pathology. This is supported by evidence that mutations in the presenilin 1 (PSEN1) gene are the major cause of early-onset familial Alzheimer's disease (FAD).

Despite extensive research, the role of these molecules is not fully understood and described. To contribute to that field, in the current work we used the metabolomic fingerprint to describe the changes linked to the expression of specific genes, by monitoring fluctuations in their absence, as it is closely related to phenotype and offers an untargeted and holistic snapshot of complex biological systems. Knock out (KO) lines of the APP (APPKO) and PSEN1 (PSEN1KO) genes were produced in SK-N-SH, a human neuroblastoma cell line and model of various neuropathologies. Additionally, as the α-synuclein (SNCA) protein is involved in virtually all degenerative disorders, the SNCA gene was targeted to differentiate between general and AD-specific neurodegeneration. The findings were compared to a control line that underwent the same processing but retained all genes intact. Polar metabolites were extracted from the transformed cell lines and their high-resolution ^{1}H 1D NMR metabolomic profile was acquired on a 600 MHz spectrometer.

In depth analysis of the recorded spectra resulted in the identification of 30 metabolites including amino acids and their derivatives, organic acids, nucleotides, and amines. Significant changes in the intracellular metabolome were observed, including a reduction in glutathione (reduced form) across all cases, showing malfunction in the important antioxidant defense mechanism of the cells. Additionally, a reduction in acetate levels noted in all cases could indicate disruption functions such as gene expression, histone acetylation and lipid metabolism. Specifically, in the APPKO and PSEN1KO cell lines glutamine and glutamate levels decreased. Dysregulation in the glutamine-glutamate cycle has been noted in various neurodegenerative diseases and could present interesting therapeutic targets in the future. Interestingly, lactate levels also dropped in the APPKO and PSEN1KO lines, pointing to changes in the energy production and glycolysis cycle in neuronal cells with these malfunctioning genes.

The findings of this study could shed light on possible intercellular indicators and provide complementary information on the causes, pathology and progression of the disease.

References

[1] Li Y, Bohm C, Dodd R, Chen F, Qamar S, Schmitt-Ulms G, Fraser PE, St George-Hyslop PH. Structural biology of presenilin 1 complexes. Mol Neurodegener. 2014 Dec 18;9:59. doi: 10.1186/1750-1326-9-59. PMID: 25523933; PMCID: PMC4326451.