In vitro metabonomic study of metabolic changes generated by APP gene expression

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1. Introduction

Alzheimer's disease (AD), which is a progressive and incurable neurodegenerative disorder, is the most common age-related disease and human dementia in the world [1]. The clinical signs include loss of memory and language and behavioral disorders affecting reasoning, judgment and mood. One of the main cause of this disease is the formation in the neuronal extracellular environment of senile plaques composed by the β-amyloid peptide [2]. Therefore, the APP gene responsible for β-amyloid peptide production appears as an interesting target in the case of Alzheimer's disease research.

2. Approach

In order to assess the effects of APP gene expression on metabolism, the metabolome of neuroblastoma cells in which this gene is transfected (SH-SY5Y APP) has been compared with the metabolome of "control" neuroblastoma (SH-SY5Y). This makes it possible to highlight possible metabolic changes induced by APP gene expression in physiological conditions.

By using a metabonomic-based approach, the intra and extracellular media of each cell line were analyzed by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The intracellular media has been obtained by a methanol: water:chloroform extraction. The culture medium in which the cells grew was analyzed to reflect the modification that has occurred in the extracellular media.

3. Results

Principal component analysis reveals important differences between the metabolome of SHSY-5Y and SHSY-5Y APP cells in polar phase (Fig.1) and in the extracellular media.

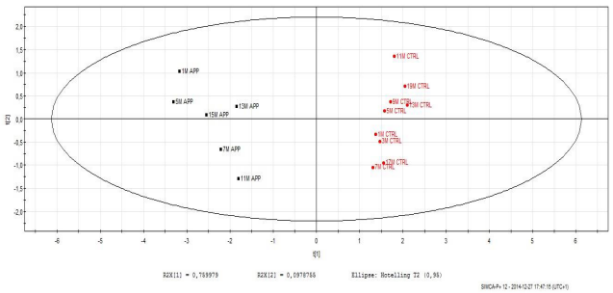

Figure 1. PCA representing separation between SHSY-5Y (red) and SHSY-5Y APP (dark) polar phases

In the SH-SY5Y intracellular media obtained by methanol:water:chloroform extraction, the main metabolic disturbances observed in the polar phase are decreases in isoleucine (δ=3,69ppm), choline (δ=3,25ppm) and glutamate (δ=2,12 – 2,36 – 2,44 – 3,77ppm) concentrations. Increases of lactate (δ=4,13ppm), myo-inositol (δ=4,09ppm) and UDP-glucose (δ=4,17 – 5,96ppm) are also observed.

Metabonomics approach also showed that the extracellular media was modified differently by both cell lines. In fact, levels of lactate (δ=1,36 – 4,13ppm), alanine (δ=1,48ppm), glutamate (δ=2,32ppm) and acetate (δ=1,92ppm) decreased in SH-SY5Y APP extracellular media. In parallel, glucose (δ=3,25 – 3,41ppm), leucine (δ=0,96 – 3,73ppm), α-cétoglutarate (δ=2,44ppm) and glutamine (δ=2,12 – 3,77ppm) increased.

4. Discussion

Important metabolic disturbances were highlighted by in the SHSY-5Y APP metabolome as compared to control neuroblastoma cell line both in the extracellular and intracellular compartments.

Energetic pathway as well as glutamate and acetylcholine productions are altered in SHSY-5Y APP cells, indicating a weakness already existing in those cells due to the presence of APP gene.

References

1. Frozza, Rudimar L., Mychael V. Lourenco, and Fernanda G. De Felice, 'Challenges for Alzheimer's Disease Therapy: Insights from Novel Mechanisms Beyond Memory Defects', *Frontiers in Neuroscience*, 12 (2018)
2. Kumar, Anil, Arti Singh, and null Ekavali, 'A Review on Alzheimer's Disease Pathophysiology and Its Management: An Update', *Pharmacological Reports: PR*, 67 (2015)