Multiomic Signature in Human Neural Models

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The human brain is a very complex organ with unique characteristics, which makes it the target of many studies aiming to investigate it in its entirety. In this context, human neural models emerge as alternatives to in vivo models for the study of the central nervous system in vitro. Following this path, three neural models were selected (a 2D culture of SH-SY5Y cells, a 3D model with 3- and 10-day neurospheres of the GM23279A cell line, and 45-, 60-, 75- and 90-day organoid models of the GM23279A cell line, and also a lipid dataset (25-week cortical brain organoids) to perform a multiomic signature, integrating proteomics, lipidomics, and metabolomics. The objective is to characterize the Global Proteome, performing an enrichment analysis of terms related to neurogenesis, as well as identifying specific markers for each cell type.

The methodology used in the three datasets involved a shotgun proteomic approach. Each dataset had its proteins extracted, alkylated, reduced and digested with trypsin. The generated peptides were isobarically labeled with iTRAQ 4-plex. After this step, the peptides were separated offline using hydrophilic interaction chromatography (HILIC). The generated fractions were inserted, in technical replicate, into the LTQ Orbitrap Velos/Q-Exactive Plus mass analyzer. The generated raw data were processed using Proteome Discoverer version 2.3.0.

Results identified approximately 1742 proteins and 3,656 peptides in the 2D SH-SY5Y cell culture dataset. The 3D neurospheres dataset revealed 3,958 proteins and 10,139 peptides, while the cerebral organoid dataset identified 6,249 proteins and 38,584 peptides. Proteins related to axonal guidance and GTPases were found across all three proteomic datasets.

Lipidomic analysis was performed descriptively with absolute quantification in triplicate on single 25-week cortical brain organoids, quantifying more than 500 lipid species. Metabolomic analysis provided complementary insights, revealing changes in key metabolic pathways, with significant alterations in amino acid metabolism, energy production, and neurotransmitter biosynthesis.

Agradecimentos: