Protective factors in the elderly: comparative astrocytes proteome in Brazilian centenarian\'s vs critically ill adults with COVID-19

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COVID-19 has posed significant challenges globally, with Brazil being one of the hardest-hit countries by the pandemic. The emergence of the Gamma (P.1) variant of the SARS-CoV-2 virus in Brazil led to a surge in infections and fatalities. Despite the grim statistics of COVID-19-related deaths, there were notable cases of centenarians exhibiting resilience to severe illness caused by the virus. In this study, we aimed to elucidate the proteomic signatures associated with SARS-CoV-2 infection and individual factors that confer protection or susceptibility in human astrocytes derived from iPSC originated from mild infected centenary patients (n=4, CENT) and critically ill adults (n=3, ADU). Our analysis was focused on the Gamma (GAM) and Wuhan (WU) variants, comparing the response to infection across two age groups. Each patient-derived sample in the study has three conditions: cells infected with WU and GAM variant of SARS-CoV-2 virus, or the non-infected cells used as Control (CTRL) for 21 samples in the cohort. Proteins were extracted and STrap digested, the LC-MS/MS were performed using a LFQ and DIA mode acquisition. A principal component analysis was performed and the first component accounted for most of the variability and differentiated infection from CTRL, but in the second component, it was not possible to differentiate between virus type or age. DIA-MS analysis of astrocyte cells identified 6054 proteins and 3096 were quantified for each comparison group. The dynamic range of the quantified proteins spanned over five orders of magnitude, with the top 30 proteins contributing to around 34% of the total abundance. A total of 19 astrocyte markers corresponding to metabolic, structural, and membrane proteins were identified. Expression of receptor NRP1, involved in astrocyte infection by SARS-CoV-2, was up-regulated in all infected groups, but interestingly was found with a higher fold-change in ADU rather than CENT. Differential protein expression analysis was conducted to compare viral infection vs CTRL for each age. A GOBP functional analysis with the Funrich tool for the significantly changing proteins shows that the energy pathways, protein metabolism, and metabolism were the most enriched among the four groups. Next, we quantitatively compare CENT vs ADU within the GAM or WU group by a fold-change enrichment analysis using the significantly changing proteins previously detected in each virus vs CTRL. Within GAM or WU infection, ADU exhibited enrichment in the metabolism of fatty acid, lipids, and xenobiotics; energy processes (electron transport, mitochondrial transport), proteolysis/peptide lysis, and inflammatory/ immune response, while CENT was more enriched in peptide/amino acids metabolism, cell organization/ migration/ differentiation, DNA replication/repair, gene silencing, and epigenetic regulation of gene expression. Processes found in ADU agree with several studies showing an association between the metabolic imbalance of amino acids, lipids, and energy-

generating pathways with disease severity and critical outcomes in COVID-19. While in CENT, cellular processes, DNA repair, and epigenetic regulation of gene expression could be explaining or contributing to their resilience against severe COVID-19. Overall, our findings provide valuable insights into the molecular mechanisms underlying COVID-19 pathology and the differential response of astrocytes to infection in mild centenarian vs critically ill adults.

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