Spatial proteomic analysis of Congenital Zika Syndrome development

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Studying Congenital Zika Syndrome (CZS) in animal models such as mice is crucial for understanding how the Zika virus (ZIKV) affects brain development. Although congenital Zika syndrome (CZS) is characterized by severe microcephaly, there are also mild cases of CZS that need to be studied, as the literature on them is scarce. These studies could provide insights into ZIKV pathogenesis in the brain during infections in later pregnancy stages. In this context, *in vivo* models are more flexible for studying different infection timelines. Our work focuses on the studying a murine model, which represents late ZIKV infection. By examining specific brain regions, we aim to uncover common or distinctive molecular signatures of each region and understand the impact on brain function. A neonatal mouse model was used to study mild CZS development. The mice were infected on postnatal day 0 (P0) with the Brazilian ZIKV (Recife/Brazil, ZIKV PE/243, accession no: KX197192.1) or control (MOCK) was injected into the lateral ventricle of the brain with a dose of 30 PFU. Brain regions (i.e., cortex (CX), diencephalon (DI), brainstem (TR), cerebellum (CB), bulb (BL), hippocampus (HC), and hypothalamus (HT)) were collected at postnatal day 10 (P10). We performed a bottom-up proteomic approach involving brain protein extraction in lysis buffer with 10% SDS and 100 mM TEAB, delipidation, reduction/alkylation, and tryptic digestion with S-Trap methodology. Tryptic peptides were analyzed using label-free quantification and DIA mode acquisition. DIA-NN software with an *in-silico* library from *Mus musculus* was employed for protein identification and quantification. A total of 9,233 protein groups were identified. In Principal Component Analysis, a higher separation was observed in the first component for ZIKV and MOCK groups. Furthermore, CB and BL brain regions were the more diverging ones, and the rest clustered more tightly. Student t-test statistical analysis ($p<0.05$) was used to determine the differentially abundant proteins (DAP) in each brain region, comparing ZIKV vs MOCK groups. It detected 3,505 statistically abundant differential proteins in BL, 3,678 in CB, 2,775 in CX, 2,384 in DI, 3,140 in HC, 1,737 in HT, and 360 in TR. Functional enrichment of DAP from ZIKV vs. MOCK was performed for GOBP and KEGG pathways using the String platform. Glutamatergic synapse was detected in all brain regions, whereas GABAergic synapse was exclusive to the BL, CB, DI, and HC regions. Distinct enriched processes were identified in some brain regions: the BL region showed regulation of synaptic plasticity, neurotransmitter levels, and the synaptic vesicle cycle; the TR region exhibited dopaminergic synapse; and the HT region displayed axon guidance. Processes related to synapse and neuron development were enriched across all brain regions. GABAergic synapses are limited to specific brain regions, suggesting distinct

regulatory mechanisms of inhibitory signals. Unique processes in the BL, TR, and HT regions reveal their specialized roles in synaptic regulation and neuronal development. Additionally, the widespread enrichment of synapse and neuron development processes highlights the massive impact of ZIKV infection on overall brain function. The alteration of brain synapse formation can be related to cognitive disorders experienced by infected fetuses.

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