Axonogenesis impairment in mouse models of severe and mild Congenital Zika Syndrome

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Congenital Zika Syndrome (CZS) is caused by vertical transmission of the Zika virus (ZIKV) during pregnancy, leading to various pathologies in affected fetuses. Although global ZIKV outbreaks have diminished, CZS remains a significant concern in endemic countries. Nowadays, CZS severity studies remain unexplored. To address this gap, we conducted a comprehensive study using *in-vivo* mouse models and PTMomics to investigate the molecular and signaling mechanisms underlying both severe and mild forms of CZS. Our approach focused on examining alterations in the proteome, N-glycoproteome, and phosphoproteome caused by infections during different gestational periods (model for the second and third trimesters of human pregnancy). Brain proteins were digested using the S-Trap methodology. For the enrichment of N-glycopeptides and phosphopeptides, Zic-HILIC and TiO_2 spheres were employed, respectively. N-glycopeptides were deglycosylated with PNGase-F in O^{18} water. Additionally, a fraction of intact N-glycopeptides was analyzed to identify glycan composition. For statistical analysis, each ZIKV infected group was compared with its corresponding control (MOCK). Through label-free proteomics, N-glycoproteomics, and phosphoproteomics analyses, we detected 5,062 and 5,000 proteins in severe and mild CZS, respectively. In addition, 934 and 888 N-glycoproteins, 1,030 and 979 phosphoproteins, 1,668 and 1,488 N-glycosites, and 2,245 and 2,176 phosphosites were identified, corresponding to severe and mild CZS, respectively. Morphological processes were negatively affected in both severe and mild Congenital Zika Syndrome (CZS). Axonal development was extensively disrupted in mild and severe CZS, with early axonogenesis processes, such as site polarization and microtubule polymerization, being impaired. This impairment was reflected in the decreased abundance of tubulin and microtubule-associated proteins. Additionally, final axonogenesis processes were also altered, including growth cone formation and microtubule ending, especially in severe CZS. The inhibition of axonal growth cone seems to be regulated by phosphorylation, with GSK3? kinase inhibition playing a role in severe CZS and CDK5 kinase inhibition in mild CZS. Another morphological structure that was altered was the dendrites, particularly in severe CZS, which was regulated by phosphorylation. In addition, histological examination revealed decreased dendritic arborization of pyramidal neurons in postnatal ZIKV infection. These findings demonstrate that ZIKV can cause neurological damage even at advanced stages of neurogenesis. The PTMomics strategy revealed that axonogenesis impairment is mediated by disruptions in microtubule structure, growth cone, and microtubule endings. Our study highlights molecular mechanisms by which ZIKV affects neuronal development, providing valuable insights about CZS severity.

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