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Mentor: Michal Hetman, M.D., Ph.D.

Title of project: Neurotoxicity of Zika Virus Protein C is Mediated by Nucleolar Stress

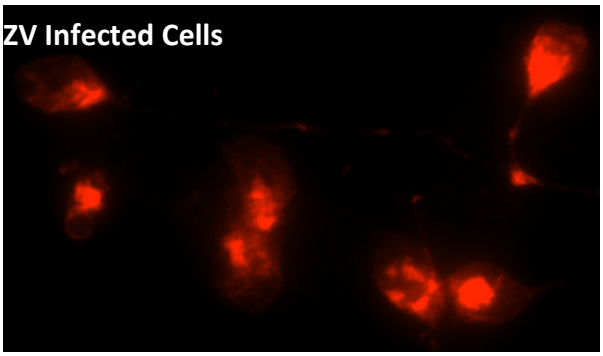
Microcephaly is a component of several rare neurodevelopmental syndromes that are due to mutations of genes that are involved in such processes as chromatin structure, microtubule organization, RNA splicing, ribosomal biogenesis or lipid synthesis. In addition, microcephaly may be a consequence of viral infections during pregnancy. Mechanistically, microcephaly is caused by insufficient neurogenesis due to loss of neuroprogenitor cells and/or their impaired proliferation and/or loss of their neuronal progeny. In consequence, microcephalic individuals are often disabled including persistent cognitive deficits. Zika virus (ZV) is a small RNA virus that belongs to the Flaviviridae family of arthropod-borne viruses. During the recent Zika epidemic in South America, a disturbing association has been suggested between ZV infection during pregnancy and microcephaly. However, pro-microcephalic potential of ZV has never been examined in the laboratory. The nucleolus which is a center of ribosomal biogenesis is prominently present in many types of neurons as well as rapidly proliferating cells. Perturbation of any step of ribosomal biogenesis triggers ribosomal stress (RS) that suppresses cell growth and/or induces apoptosis in a p53-dependent or p53-independent manner. While in proliferating cells the RS is a major tumor suppressor mechanism, it is also proposed as a contributing factor to neurodevelopmental diseases that include microcephaly. Interestingly, many viral proteins that interact with RNA have an affinity towards the nucleolus. In such a location they may disturb activity of ribosomal biogenesis factors and activate RS. Indeed, a capsid protein of a close relative of ZV, West Nile Virus, has been shown to localize to the nucleolus, induce RS and the p53-mediated cell death. The hypothesis to be tested during this summer research project is that ZV capsid protein enters nucleolus of neuroprogenitors/immature neurons, activates RS, and, in consequence, triggers p53-mediated cell death. The following studies have been performed to test such a possibility:

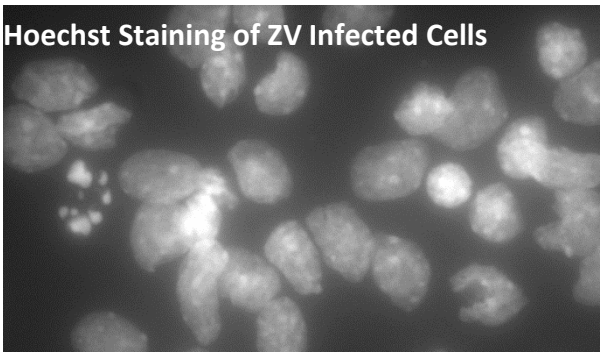
Study 1. Fluorescent imaging to determine subcellular distribution of the overexpressed ZV capsid protein in primary cortical neurons from rat embryos and newborn pups.

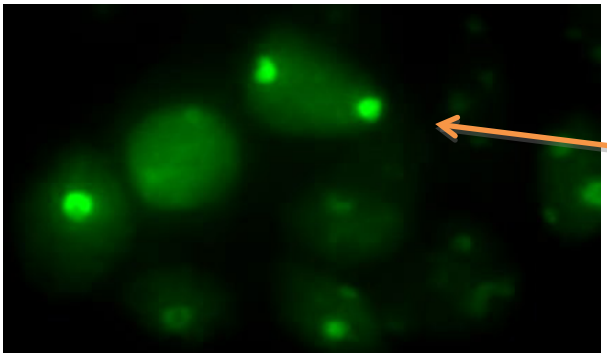
Study 2. Study structural integrity of the nucleolus in neurons that overexpress ZV capsid protein.

Study 3. Investigate the potential role of RNA binding regions in the capsid protein by mutating specific amino acid residues and comparing the neurotoxicity to the wild type and structural protein M.

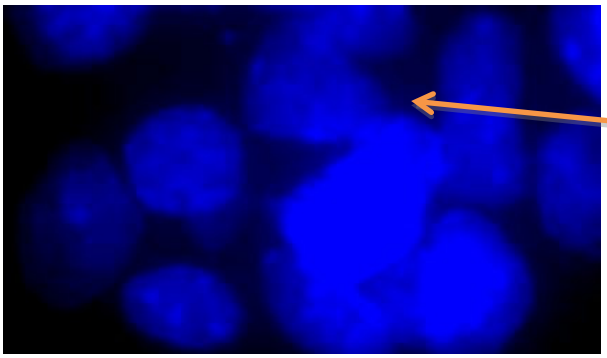
Study 4. Determine effects of the live ZV and ZV capsid protein on neuronal survival.







B23 Staining of Cells Transfected with Plasmid containing Capsid Protein



Hoechst staining from Cells Transfected with Plasmid containing Capsid Protein.