Varicella zoster virus (VZV) is a neurotropic alphaherpesvirus. During primary infection, VZV causes varicella (chicken pox), after which the virus go latent in ganglionic neurons along the entire neuraxis before reactivating decades later to cause zoster (shingles). Interferon gamma (INFγ), produced during viral infection, stimulates transcription of genes that mediate antiviral responses. Herein, it was tested whether INFγ treatment of human neurons inhibits VZV infection of human neurons in vitro. Infected neurons not treated with INFγ developed a cytopathic effect in 4 weeks, during which time VZV DNA increased 7-fold and viral RNA accumulated. Infected neurons cultured in the presence of INFγ for 8 weeks or infected neurons cultured in INFγ for 4 weeks followed by cytokine removal for an additional 4 weeks had only a 2.8- and 3.6-fold increase of viral DNA, respectively in the 8 weeks post-infection. Furthermore, levels of VZV transcripts did not increase between 4 and 8 weeks post-infection when INFγ was removed at 4 weeks post-infection, and even began to decrease when the cultures were maintained in INFγ for the entire 8 weeks. In accordance with reduced DNA accumulation and mRNA levels when infected neurons were maintained in INFγ, less CPE was evident at 8 weeks post-infection compared to cultures which had INFγ removed at 4 weeks post-infection. Replication of VZV DNA and transcription of viral genes was inhibited by INFγ, and the extent of virus gene expression in INFγ-treated neurons compared to VZV expression in latently infected human ganglia remains to be determined.

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The Retroviral Cyclin Controls CDK8-mediated Transcription Elongation and Reinitiation

Claire H. Birkenheuer *, Connie D. Brewster, Sandra L. Quackenbush, Joel Rovnak

Department of Microbiology, Immunology, and Pathology, Colorado State University

Wall-eyed dermal sarcoma virus is a complex retrovirus that causes seasonal tumors in wall-eye fish. RV-cyclin is one accessory protein encoded by the virus, and is one of only two viral proteins expressed during tumor development. Therefore, role of RV-cyclin in tumor development was explored. RV-cyclin interacts with host cyclin dependent kinase8 (CDK8). CDK8 has oncogenic like properties in colon cancer and melanoma, and one target of CDK8 kinase activity is the carboxy terminal domain of RNA Pol II. Here qRT-PCR analysis demonstrates RV-cyclin’s direct interaction with CDK8 increases transcript levels of another set of oncogenes—the serum-response genes (Fos, EGR1, and jun). Nuclear run-on experiments, and chromatin immunoprecipitation experiments with an antibody to RNA Pol II, show that RV-cyclin enhances transcription elongation along the EGR1 gene locus. This enhancement correlates with increased recruitment of CDK8 to the EGR1 gene locus. In addition to increasing CDK8 occupancy at the EGR1 gene, in vitro kinase experiments demonstrate RV-cyclin increases the amount of CDK8-phosphorylation on the CTD of RNA Pol II. In conclusion, not only does RV-cyclin direct CDK8 to specific genes during tumor development, RV-cyclin enhances CDK8 kinase activity while it is there. The end result of the CDK8-RV-cyclin interaction is a rise in the mRNA levels of another pool of oncogenes, the serum-response genes. This is one mechanism by which RV-cyclin could contribute to the development of wall-eyed dermal sarcoma.

*Correspondence to: Department of Pathobiological Sciences, Louisiana State University

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Predicting Prion Propensity of Human Proteins

Cascarina S, Ross E.

Department of Biochemistry and Molecular Biology, Colorado State University

In humans only a single prion-forming protein named PrPc (for “cellular prion protein”) is currently known, yet many more neurodegenerative disorders involve aberrant protein aggregation. The classical model for these diseases has involved cell-autonomous aggregation, assuming that aggregation occurs independently in each cell within a diseased patient. However, more recent models have proposed a non-cell-autonomous progression of disease in which aggregates formed in one cell may be transmitted to neighboring cells. These aggregate seeds then cause aggregation of the soluble protein in the “infected” cells, similar to the prion diseases. Within the past few years, a number of proteins that exhibit prion-like aggregation and spread to neighboring tissues have been discovered in patients with Amyotrophic Lateral Sclerosis (ALS). Although ALS has been studied for a number of decades, these proteins were only recently linked to ALS by chance. This demonstrates a clear need for an accurate method to systematically identify additional proteins that may play a pathological role in neurodegenerative disorders. Taking advantage of the compositional similarity of these proteins to the known yeast prions, I plan to use the prion prediction methodology that our lab has pioneered to develop an entirely new algorithm specifically suited for this class of neuronal proteins.

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Ivermectin for the Control of West Nile Virus Transmission

Nguyen C, Burton T, Kuklinski W, Gray M, Foy BD

Department of Microbiology, Immunology, and Pathology, Colorado State University

Presently there are limited options for controlling the transmission of West Nile virus (WNV), including the use of larvicides and adulticides to target the mosquito vector. However, these methods are poorly-targeted, restricted to wealthy semi-urban and urban areas that are able to fund the efforts, and opposed in some communities due to toxicity concerns. This study evaluated the use of endectocide-treated bird feed to control WNV transmission by targeting the primary vector in Colorado, Culex tarsalis. Ivermectin susceptibility in C. tarsalis was first measured through ivermectin-spiked bloodmeals fed using membrane feeders, and the LC50 was determined to be 49.94 ng/ml (39.71-59.93 95% CI, n = 988). Chickens were then fed ivermectin-treated feed to examine its safety and palatability, and mosquitoes were blood fed directly on the chickens to assess in vivo effects. Finally, ivermectin pharmokinetics were analyzed using vein blood from chickens as well the C. tarsalis that bloodfed on the chickens. A mixture of 200 mg ivermectin/kg of bird feed was determined to be a palatable and safe dose on which chickens would feed while also being effective in killing C. tarsalis in bioassays. Pharmacokinetic data from the in vivo tests produced conflicting results compared to in vitro blood feeds but drug was detected in chicken blood at concentrations that may be expected to affect C. tarsalis. Dosing, safety, and bioassays are currently being conducted in doves and sparrows. Additional studies are currently determining the effect of ivermectin on mortality in WNV-infected mosquitoes, as well as if ivermectin reduces WNV replication and transmission. Our study indicates that the use of ivermectin-treated bird feed could be a novel method of controlling WNV transmission.

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Bovine herpesvirus 4 not detected in free-ranging domestic cats from California, Colorado, and Florida

Chiu E, Troyer R, VandeWoude S

Department of Microbiology, Immunology and Pathology, Colorado State University