P-5

Structure-Function Characterization of Non-Primate AAVs

Mario Mietzsch[1], Victoria E. Fielding[1], Judit Penzes[1,2], Qian Yu[2], Paul Chipman[1], Robert McKenna[1], Giovanni DiPasquale[3], John Chiorini[3], Ya Li[4], Lin Yang[4], Sergei Zolotukhin[5], Peter Tijssen[2], and **Mavis Agbandje-McKenna**[1]

[1]Department of Biochemistry and Molecular Biology, Center for Structural Biology, McKnight Brain Institute, College of Medicine, University of Florida, Gainesville, FL, USA; [2]Institut National de Recherche Scientifique - Institut Armand Frappier, Laval, QC, Canada; [3]National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD, USA; [4]State Key Laboratory of Biotherapy, Sichuan University, Chengdu, China; [5]Department of Pediatrics, College of Medicine, University of Florida, Gainesville, FL, USA

All adeno-associated virus (AAV) vectors currently used in gene therapy trials or approved human gene therapy biologics are based on primate AAVs. One hurdle for AAV vectors in human gene therapy are pre-existing neutralizing antibodies (NAb) that target virus capsids, leading to vector inactivation and to a loss of treatment efficacy. To overcome this issue the capsid surface of these AAVs can be mutated to escape from Nabs. An alternative to this strategy is the utilization of AAVs that do not exist in the primate population, but have the ability to infect mammalian cells and exhibit low or no reactivity with human sera. Here we present the capsid structures of bovine AAV (BAAV), BatAAV, Bearded Dragon Parvovirus (BDPV), and snake AAV (SAAV) determined by cryo-electron microscopy to 2.6, 3.1, 3.2, and 3.1 Å resolution, respectively. These viruses show very low capsid sequence identities (~51-59%) to AAV2, other primate AAVs, and each other, as has been reported for the sequence and structurally diverse AAV4 and AAV5 serotypes. While the core structure of the capsids of these non-primate AAVs are conserved, their surface loops display unique structural features different to the primate AAVs and each other. These differences are located in the previously defined capsid variable regions. Significant structural differences include a large insertion in VR-V of BAAV, large insertions in VR-III and VR-VII of BatAAV, and distinct truncations of VR-IV of BatAAV and SAAV. Many of the surface loops have previously been identified as binding sites for NAbs in the primate AAVs. Native immuno-dot-blots confirm the lack of antigenic reactivity of BAAV, BatAAV, BDPV, and SAAV towards different anti-AAV antibodies or human sera. The structure of these viruses in addition to their cell binding and transgene expression phenotypes will be presented.