Characterization of Assembly-Activating Protein in Adeno-Associated Virus Capsid Assembly

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Great advancements have been made in using adeno-associated virus (AAV) vectors for gene therapy; however, the basic biology behind AAV capsid assembly remains poorly characterized. Discovery of assembly-activating protein (AAP), which promotes AAV capsid assembly, provides a new method for studying AAV capsid assembly. Recent studies reveal that not all serotypes of AAV require AAP for capsid assembly, and that a single mutation capsid protein of AAV11 (AAP-independent) or AAV12 (AAP-dependent) can alter their AAP-associated phenotype This project aims to extend existing research results and computational tools through developing and testing a hypothesis on identifying the crucial region that potentially regulates AAP dependency, to serve as an initial step towards a multidisciplinary methodology for viral vector studies. Amino acid residues potentially regulating AAP dependency were identified through a combination of DNA sequence alignments and computer models of the tertiary and quaternary structure of several AAV serotypes. These residues were then mutated to the heterologous amino acids in AAV11, cloned into the wild type plasmid of AAV2, -8, and -9 (AAP-dependent), transfected into cells, and evaluated for AAP dependency. The introduced mutations did not confer a gain of AAP-independent capsid assembly function but instead showed structural deformities that prevented capsid assembly. A computer algorithm that predicts capsid assembly deformities, based on a distance metric between residues and a sliding window to comprehend the structural impact on neighboring residues, was created to help exclude disruptive mutations in future experiments. Initial results show good correlation between in silico and in vitro data.

Awards Won:
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