Visualizing the Adeno-Associated Virus Vector through Modification of the Viral Protein 2

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Adeno-Associated Virus (AAV) is a non-pathogenic virus that has become a popular vector in gene therapy. To date, hundreds of AAV variants have been researched, however their passage into the nucleus and transduction mechanism is incompletely understood. The research objective was to create a modified AAV vector with surface-bound enhanced green fluorescent protein (EGFP) using modified VP2 capsid protein from AAV2, 2VP2. AAV formation with 2VP2-EGFP would be attempted with multiple viral serotypes, each with its own cellular tropism. A set of plasmids including the engineered pEGFP-2VP2 plasmid and a luciferase reporter gene were transfected into HEK293 cells to produce an AAV viral vector. The modified AAV was retrieved, purified and transduced into HeLa cells. These procedures were successfully performed to produce at least six different serotypes with surface-bound EGFP-2VP2, which were confirmed by luciferase assays and western blot analyses. Confocal fluorescent microscopy was used to visualize intracellular AAV3-2VP2-EGFP location. Additionally, there was not a significant difference in production or infectivity of the traditional and EGFP-2VP2-modified AAV2 and AAV3 vectors. The research findings have several therapeutic implications. Creation of AAV vectors with surface-bound GFP may facilitate intracellular visualization and further understanding of AAV transduction, enabling more effective AAV-based therapy, as vectors could be engineered according to how AAV passes through the cytoplasm and nuclear envelope. Capsid-bound GFP takes no genetic space within AAV, maximizing capacity for larger therapeutic genes. The successful creation of multiple AAV serotypes with modified 2VP2-EGFP also broadens the applicability of therapy to various tissues and illnesses.

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