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Systemic Evaluation of Host and Viral Elements Affecting AAV Encapsidation

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Adeno-associated virus (AAV) has been developed as a safe and efficient gene therapy platform which has shown great promise in human clinical studies and pharmaceutical development. Nevertheless, the broad application of AAV vectors in gene therapy can not be achieved until it is no longer a challenge task to produce high quality AAV economically. Here, a systemic approach to develop an AAV packaging strategy in mammalian cells using a vaccinia carrier was reported. First, all AAV helper genes are integrated into one vaccinia carrier. Second, a new production cell line has been developed for maximal production efficiency. In this new system rAAV vector can be produced in mammalian cells without tedious transfection procedures or wild type AAV revertant generation. For quality control, vector integrities were studied using a high throughput genomic approach. Empty particles have always been generated as impurities during AAV vector production, the biological properties of three types of "empty" AAV particles were evaluated: syngeneic pseudo-vectors with partial AAV genomes derived from DNA of the corresponding full particles, allogeneic pseudo-vectors with partial genomes different from the corresponding full particles, and null pseudo-vectors with no DNA inside the capsids. The syngeneic particles in excess increased the corresponding full AAV vector transgene expression both *in vivo* and *in vitro*. However, such effects were not observed with null or allogeneic particles. The observed differences among these pseudo-AAV particles may be ascribed to the syngeneic pseudo-vector DNA facilitating the complementary DNA synthesis of the corresponding full AAV particles. In addition, the issue of capsid glycosylation has also been analyzed. In summary, a wide range of topics related to AAV encaspidation have been studied. Their potential implications for future clinical application will be discussed.