RATIONAL DESIGN OF SPLIT GENE VECTORS TO EXPAND THE PACKAGING CAPACITY OF ADENO-ASSOCIATED VIRAL VECTORS.

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Abstract

Adeno associated viruses (AAV) have recently been demonstrated as a very promising gene delivery vehicle. But the limited packaging capacity of AAV vectors (~4.7kb) hinders their application for diseases involving large genes such as those responsible for Duchenne muscular dystrophy and cystic fibrosis. To overcome this hurdle, the trans-splicing and overlapping dual vector methods were developed to expand the packaging capacity of AAV. It has been demonstrated that certain expression limiting barriers affect transduction from these dual vectors. The trans-splicing method requires an optimal gene splitting site and the overlapping method requires a highly recombinogenic domain in the middle of the gene for high levels of transduction. To overcome these limitations of dual vectors, we developed a novel trans-splicing/overlapping hybrid vector system that can efficiently reconstitute any large gene. The experimental data demonstrate that the hybrid vector system improves gene expression compared to the traditional dual vectors. The study also demonstrates that the rationally designed trans-splicing AAV vectors can be successfully used for body-wide gene delivery. Taken together, this study outlines the considerations to be taken into account for rational design of split gene vectors that would be capable of efficient transgene expression.