O-21

Capsid Protein of Various AAV Serotypes are Involved in the Transcription of Viral Genome

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Introduction: The amino acid Y704 in adeno-associated virus serotype 2 (AAV2) capsid is near the 2-fold interface. Previous researches reported that mutation of this surface-exposed tyrosine residue to phenylalanine (AAV2-Y704F) could modestly increase the recombinant AAV2 (rAAV2) vector-mediated transgene expression; conversely, the mutation of the same position to an alanine residue (AAV2-Y704A) was defective for transcription in the presence of adenovirus. This study, we would like to characterize whether the mechanistic roles of the Y704 in vector-mediated transgene expression might differ among AAV serotypes in the absence of helper adenovirus.

Methods and Results: By site-directed mutagenesis, we generated pairs of point mutants on various AAV serotypes: AAV2-Y704A, AAV2-Y704F; AAV3-Y705A, AAV3-Y705F; and AAV6-Y705A, AAV6-Y705F. It was concluded that all the mutant capsids encapsidate viral genomes at a similar level. Then, in vitro cell binding and internalization assays were performed by quantitative PCR assays. Our results demonstrated that similar to AAV2, all other mutant capsids have little effect on viral binding and internalization ability. In terms of intracellular trafficking, Y to A mutants had a similar ability compared to the wild-type capsid, whilst Y to F mutants appeared to increase it. On the other hand, all the Y to A mutants dramatically shutdown the mRNA transcription and hence the transgene expression in vitro, whilst most Y to F mutants significantly increased both the mRNAs and proteins. To our surprise, we found that the defective effect of Y to A mutants on transgene expression is not only in cis manner, but also in trans. Further mechanistic studies indicated that the inverted terminal repeat (ITR) of viral genome might play a significant role. Next, an additional pair of viral vectors, AAV8-Y707A and AAV8-Y707F will be generated to test our hypothesis in mice liver *in vivo*.

Conclusion: We have characterized the biological properties of Y704 of AAV capsids and concluded that they are conservative among AAV serotypes. Interestingly, the Y to A mutants failed to accumulate mRNA transcripts in the presence of significant levels of viral ITR-containing DNAs in the nucleus.