

level of expression 2 weeks-post injection. Such distinct expression kinetics depending on the route of vector administration was not observed with rAAV8 vector. In the second experiment, we injected C57BL/6 mice with rAAV9 vectors expressing a lacZ marker gene; i.e., AAV9-EF1 α -nls lacZ or AAV9-CMV-lacZ via TV at two different doses, 3.0x10¹¹ and 1.8x10¹² vg/mouse (n=2 per group, total 8 mice for 4 groups). Development of anti- β galactosidase antibody was monitored by ELISA. The antibody was not detected in any of the 8 mice 7 days post-injection, but detected in 3 of 4 mice injected with AAV9-CMV-lacZ 10 days post-injection, when all the 8 mice were sacrificed for tissue distribution analysis. Liver transduction efficiency with the CMV vector was 3% at 3.0x10¹¹ and reached 90% at 1.8x10¹² vg/mouse. The tissue distribution pattern with rAAV9 vectors was similar to that with rAAV8 vectors, in that, in addition to the liver, they transduced heart, skeletal muscle and pancreatic acinar cells with high efficiency. Of note is that rAAV9 had greater transduction efficiency in the heart than rAAV8, and 3.0x10¹¹ vg/mouse was enough to transduce all the cardiomyocytes via TV injection of rAAV9. Thus, rAAV9, as well as rAAV8, is a robust vector for gene therapy applications.

505. Analysis of AAV Genome Conversion and Stabilization

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Recombinant adeno-associated virus (rAAV) vectors have been widely used to deliver a variety of therapeutic genes in both in vitro and in vivo experiments. The administration of AAV vectors usually gives rise to persistent transgene expression. However, the doses required for meaningful gene expression are usually much higher than other viral vectors. Because AAV genomes are single-stranded DNA (ss), we examined the conversion and stability of AAV genomes in both tissue cultured cells and in vivo animal experiments. The special AAV vectors labeled with BrdU molecules were generated and an anti-BrdU antibody was used to specifically track free ss AAV DNA genomes. Although single stranded AAV genomes were detected by conventional Southern Blot analysis, the antibody tracking, which recognizes ssDNA labeled with BrdU but not double stranded (ds) DNA with BrdU, revealed no free ss DNA molecules after AAV transduction. We concluded that ss DNA accumulation is not an essential step for transduction. In addition, we observed significant AAV genome losses during AAV transduction. Overall, less than 0.1% of AAV genomes survive the degradation in the stages of intracellular trafficking, ss DNA to double stranded DNA conversion, and ds DNA to final stabilized forms. Through a quantitative analysis of newly synthesized ds AAV genomes, we discovered that more than 90% of these molecules did not persist although they were fully functional in expressing transgenes (such as lacZ or GFP) in both tissue culture cells and mouse liver. The long term expression of transgene products observed in vivo was therefore coming from a small amount of stabilized AAV genomes. Our results suggest that improving the AAV genome stability post transduction is critical for enhancing AAV transduction efficacy.

506. Transductional and Genome Biodistribution of Novel AAV Serotype Vectors after Systemic Delivery and Their Liver Specific Dose Response Profiles in Mice

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Recent discovery of a novel family of primate AAVs led to the development of vectors with unprecedented efficiency in liver. To search for other potential applications of these vectors, we treated C57BL/6 mice intravenously with either 1x10¹¹ or 1x10¹² GC/mouse of AAVs 2, 5, 7, 8 and 9CBnLacZ. At days 3, 30 and 90, 13 tissues from treated animals were analyzed for LacZ expression by X-gal histochemical staining and vector genome quantification by TaqMan PCR. Analysis of day 3 samples revealed that AAVs 7, 8 and 9 derived vectors displayed more robust and broader tissue transduction profiles. Liver, cardiac and diaphragm muscles were the primary transduction targets by the 3 vectors. At the dose of 1x10¹¹, more than 90% of hepatocytes were transduced by AAVs 8 and 9, 60-70% by AAV7. However, at a 10-fold higher dose, 100% of hepatocytes in all 3 vector groups stained blue. In the low dose groups, AAV9 transduced cardiac myocytes the best, followed by AAV8 and AAV7. But all 3 vectors achieved remarkable transduction in the heart at the high dose. All 3 novel vectors resulted in significant numbers of X-gal positive myocyte nuclei in the diaphragm muscle, particularly at the high dose. Hepatocyte transduction achieved by AAV 2 and AAV5 were only a few percent at the low dose but a clear dose response was observed in AAV2 high dose animals. No transduction events were detected in heart and diaphragm sections of the AAVs 2 and 5 treated animals. In addition, X-gal stained muscularis cells and smooth muscle cells of some tissues in GI track including stomach, small bowel and colon were readily noticeable in AAVs 7, 8 and 9 but not in the AAV2 and 5 groups. X-gal positive stromal cells but not follicles in the ovary were occasionally visible in all vector and dose groups. Several cell types including renal tubular cells in kidney were sporadically found to be transduced by AAVs 7, 8 and 9. These 3 vectors also transduced some acinar cells, interlobular connective tissues but rarely islets in pancreas. With all vector and dose groups, no transduction events were recognized in the brain, anterior tibialis muscle and lung tissues. In contrast to distinguished tissue transductional profiles of AAV serotype vectors, there was no serotype specific genome targeting observed. In most of tissues analyzed, genome copies of all rAAVs in a particular tissue are quite similar, suggesting that the capsid directed genome processing pathway represent the determining factor for the serotype specific vector biology.

Dose response profiles of liver specific transduction by AAV1, 2, 5 and 8AlbA1T and different dosing regimens were analyzed. Our data demonstrated that, within a dose range of 1 to 30x10¹⁰ GC/mouse, all rAAVs expressed hA1AT at the levels relatively proportional to the vector dose escalation in different strains of mice. Other than being informative for identifying new applications of novel AAV vectors, the data from the present study also raised safety concerns over biodistribution of vector genomes and necessity for tissue specific gene expression of vectors when delivered systemically.