

were also transduced. Intravenous administration of scAAV2/9 to late gestation fetal macaques also produced widespread gene delivery and confirmed our observations in the mice. We, and others, have shown that systemic administration of AAV2/9 crosses the blood-brain barrier and mediates efficient gene delivery to the nervous system. The data presented here highlights the promise that this vector shows for treating systemic diseases that are perinatal lethal and affect both the brain and the viscera.

372. Targeted Mutagenesis of Ubiquitin-Binding Lysine Residues on the Adeno-Associated Virus (AAV)2 Capsid Improves Its Transduction Efficiency

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It is now well recognized that hepatic gene transfer of high doses of AAV vectors predispose to a robust adaptive immune response, from the data available from hemophilia clinical trials. Thus there is a need to develop novel strategies which will allow lower doses of vectors to be used to achieve sustained phenotypic correction and limit vector related immune-toxicities. Previous studies have demonstrated the utility of mutating surface-exposed tyrosine residues on AAV2 capsid which possibly protects the vector particles from ubiquitin-mediated proteasome degradation and resulted in a significant increase in transgene expression (Zhong *et al*, 2008). We hypothesized that mutations at lysine residues of AAV2 common capsid region, VP3, which are direct targets for host ubiquitin ligases, will improve its transduction efficiency. Our *in silico* analysis using an ubiquitination prediction software (UbPred, <http://www.ubpred.org/>) identified seven lysine residues (K39, K137, K143, K161, K490, K527 and K532) on AAV2 capsid which could be potentially ubiquitinated. Lysine>Arginine mutations in AAV2 Rep/Cap coding plasmid was carried out and highly purified stocks of a recombinant self-complementary AAV2 vectors expressing EGFP [scAAV-CBA-EGFP] were generated in each of the seven mutant plasmids. The physical particle titres of lysine mutant vectors was comparable to wild-type (WT) scAAV vectors ($\sim 0.5\text{-}1 \times 10^{12}$ vgs/mL), suggesting that these mutations do not affect the structure or packaging ability of mutant capsids. scAAV vectors containing WT or each of the seven lysine mutant capsids were then evaluated for their transduction potential *in vitro*. Approximately 8×10^4 HeLa or HEK293 cells were mock-infected or infected with AAV at different multiplicities of infection (MOI, 500, 2000 or 5000 vgs/cell). Forty-eight hours post-infection, transgene (EGFP) expression was measured by fluorescence microscopy and by flow-cytometry. Our results (Fig. 1) demonstrate that one of the seven mutants tested, the K532R vector, significantly increased gene expression in both HeLa (18X) and HEK 293 (9X) cells *in vitro* in comparison to WT-AAV2. This increased transduction efficacy of the K532R vector was consistent across three different MOIs tested, with an average increase of 10- fold over the WT vector. Further ongoing studies in normal and hemophilia B mice will demonstrate if the AAV2 K532R vector can also improve hepatic gene transfer, *in vivo*.

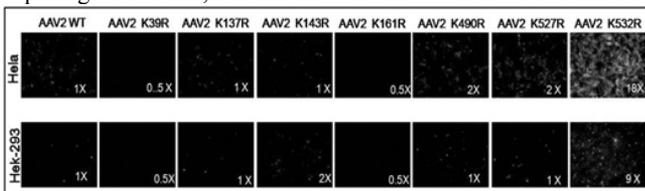


Figure 1: Transduction efficiency of AAV2 lysine mutants (MOI 2000) in HeLa and HEK293 cells *in vitro*. The relative fold-increase in gene expression is shown as inserts

Adenovirus and Other DNA Virus Vectors: Biology and Vector Design

373. Sustained and Safe Inhibition of Hepatitis B Virus Replication In Vivo Using Helper-Dependent Adenovirus Vectors To Deliver Antiviral RNAi Expression Cassettes

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Hepatitis B virus (HBV) is hyperendemic to southern Africa, east and south east Asia where there are approximately 350 million chronically infected individuals. Chronic carriers have an increased risk of developing potentially fatal complications of cirrhosis and hepatocellular carcinoma. Licensed HBV treatments rarely eliminate the virus from infected individuals, and improvement of HBV therapy remains a priority. We have previously demonstrated that CMV and U6 (Pol II/III) expression cassettes that generate artificial antiviral RNA interference (RNAi) activators can be used to inhibit HBV gene expression *in vivo*. Nevertheless, achieving safe and efficient delivery of these anti-HBV RNAi sequences remains an important objective. Recombinant adenoviruses (Ads) are amongst the most efficient hepatotropic gene delivery vehicles, but a drawback of their use is transient transgene expression and toxicity resulting from induction of host immune responses. To limit vector immunostimulation, we have generated RNAi-activating anti-HBV gutless helper-dependent (HD) Ads. Efficacy against HBV replication was tested in HBV transgenic mice, which stringently simulate the human condition of chronic HBV infection. Two days after intravenous administration of 5×10^9 recombinant HD Ads to HBV transgenic mice, 80-90% of hepatocytes were transduced. Markers of HBV replication were decreased by approximately 95% in animals receiving the HD Ads and this effect was sustained for 8 weeks without adverse effects. Compared to unmodified first generation anti-HBV Ads, inhibition of viral replication was significantly more sustained. Moreover, acute hepatotoxicity and proinflammatory cytokine release following administration of HD Ads was attenuated. HD Ad DNA was present at high concentrations in the livers of mice at termination of the investigation, which indicates that diminished efficacy was a result of switching off of transgene expression rather than elimination of the vector from hepatocytes. Alternative transcription control elements, reduction of immunostimulation by pretreatment with dexamethasone and polymer modification are being investigated to improve delivery of anti-HBV RNAi activators by these vectors.

374. Scavenger Receptor A (SR-A) and Scavenger Receptor Expressed on Endothelial Cells I (SREC-I) Are Receptors of Helper-Dependent Adenoviral Vectors

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Helper dependent adenoviral vectors (HDAds) can mediate long-term, high level transgene expression from transduced hepatocytes