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Original Article

Enhanced Transduction of Human Hematopoietic Stem Cells by AAV6 Vectors: Implications in Gene Therapy and Genome Editing

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We have reported that of the 10 most commonly used adeno-associated virus (AAV) serotype vectors, AAV6 is the most efficient in transducing primary human hematopoietic stem cells (HSCs) *in vitro*, as well as *in vivo*. More recently, polyvinyl alcohol (PVA), was reported to be a superior replacement for human serum albumin (HSA) for *ex vivo* expansion of HSCs. Since HSA has been shown to increase the transduction efficiency of AAV serotype vectors, we evaluated whether PVA could also enhance the transduction efficiency of AAV6 vectors in primary human HSCs. We report here that up to 12-fold enhancement in the transduction efficiency of AAV6 vectors can be achieved in primary human HSCs with PVA. We also demonstrate that the improvement in the transduction efficiency is due to PVA-mediated improved entry and intracellular trafficking of AAV6 vectors in human hematopoietic cells *in vitro*, as well as in murine hepatocytes *in vivo*. Taken together, our studies suggest that the use of PVA is an attractive strategy to further improve the efficacy of AAV6 vectors. This has important implications in the optimal use of these vectors in the potential gene therapy and genome editing for human hemoglobinopathies such as β-thalassemia and sickle cell disease.

INTRODUCTION

Gene therapy/genome editing of human hemoglobinopathies (β-thalassemia and sickle cell disease) is highly desirable because these are the most common human genetic diseases on earth. One out of every 600 humans globally suffers from one of these diseases. A permanent cure of sickle cell disease, the first molecularly defined human disease since 1957, is of particular significance because of the enormous physical and economic burdens worldwide. Furthermore, since the sickle mutation is among the most well-characterized, and all sickle cell patients have exactly the same mutation, the notion that if one patient can be cured, then all patients can be cured, is enormously attractive.

Although gene therapy of hemoglobinopathies was attempted, at least in animal models, with recombinant retroviral vectors more than two decades ago,[1] the low-levels of transgene expression, ranging from 0.04%–0.56%, rendered this approach less desirable. However, there has been renewed interest in this pursuit for gene therapy of β-thalassemia and sickle cell disease in recent years since the use of modified lentiviral vectors was first shown to lead to the production of potentially therapeutic levels of normal β-globin in homozygous β-thalassemic mice *in vivo*,[2] and phenotypic correction of sickle cell disease has also been achieved using modified lentiviral vectors.[3] Similar studies have been reported by a number of investigators.[4–8] The use of lentiviral vectors in clinical trials for both β-thalassemia and sickle cell disease have led to phenotypic correction of these diseases.[9–11]

However, several studies have also reported the propensity of lentiviral vectors to integrate into active genes.[12–15] Genomic sequencing of vector containing fragments from $CD34^+$ cells transduced with a lentiviral vector expressing anti-sickling β-globin showed that 86% of proviral integration occurred in genes.[7] Thus, there is a risk of insertional mutagenesis. Indeed, integration of a lentiviral vector leading to activation of a cellular proto-oncogene, HMGA2, in a patient with β-thalassemia has been reported.[9] Thus, the long-term safety of lentiviral vectors still remains to be determined.

We have had a long-term interest in the pursuit of the potential gene therapy of β-thalassemia and sickle cell disease with adeno-associated

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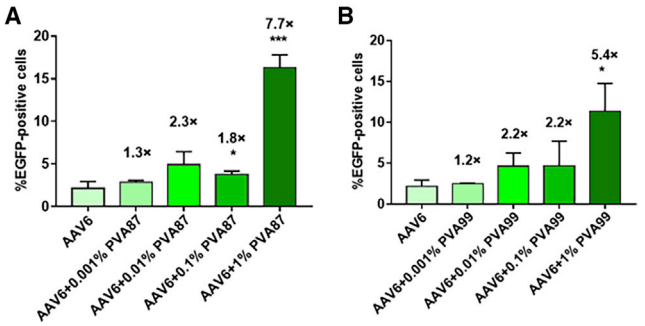

Figure 1. PVA Augments the Transduction Efficiency of AAV6 Vectors in K562 Cells *In Vitro*

(A and B) K562 cells were transduced with 1 × 10^3 vgs/cell of scAAV6-CBAp-EGFP vectors with or without pre-incubation with PVA87 (A) or PVA99 (B) at 4°C for 2 h. Transgene expression was determined by flow cytometry 48 h post-transduction. Statistical significance is indicated as *p < 0.05, ***p < 0.001.

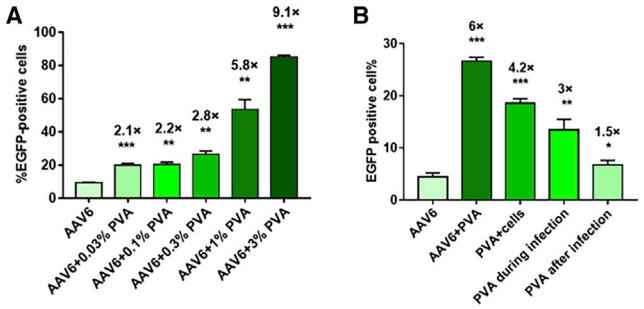

Figure 2. PVA-AAV6 Interaction Is a Critical Step in Augmenting the Transduction Efficiency of AAV6 Vectors

AAV6 vectors were pre-incubated with various indicated concentration of PVA87 and used to infect K562 cells at 3 × 10^3 vgs/cell. Transgene expression was determined by flow cytometry 48 h post-transduction as described in the legend to Figure 1 (A). The effect of PVA87 on AAV6 vector transduction was determined under various conditions as follows: (1) AAV6 alone, (2) pre-incubation of AAV6+1% PVA87, (3) pre-incubation of K562 cells with 1% PVA87, (4) addition of 1% PVA87 prior to AAV6 transduction, and (5) addition of 1% PVA87 2 h following transduction with AAV6 vectors (B). Transgene expression was determined by flow cytometry 48 h post-transduction as described above. Statistical significances are indicated as *p < 0.05, **p < 0.01, ***p < 0.001.

virus (AAV) vectors.[16] In these previous studies, while AAV2 vectors performed better (transgene expression ranging from 3%–7%) than retroviral vectors, therapeutic levels of human β-globin gene expression could not be achieved in normal and β-thalassemic mice[16,17] since AAV2 vectors do not efficiently transduce mouse hematopoietic stem cells (HSCs). In our subsequent studies, we identified AAV1 and AAV7 serotype vectors to be significantly more efficient than AAV2 vectors in transducing normal mouse HSCs, but these serotype vectors failed to transduce HSCs from sickle cell disease mice.[18–20] Thus, we concluded that mouse models of HSC transduction and sickle cell disease are not a good surrogate, at least for AAV vectors.

In 2013, we first reported the identification of AAV6 as the most efficient serotype for transduction of primary human HSCs.[21] Subsequently, three independent groups corroborated that AAV6 vectors are highly efficient in genome editing in primary human HSCs.[22–24] More recently, AAV6 vectors were reported to lead to successful genome editing of sickle mutation in primary human HSCs from patients with sickle cell disease.[25] However, multiplicities of infection (MOIs) of 100,000–200,000 vgs/cell were required to achieve transduction efficiencies ranging between 45%–55% in those studies, although different strategies have been explored to improve the transduction efficiency of AAV6 vectors in human HSCs, such as the use of (1) self-complimentary AAV vectors,[26–28] (2) tropism specific promoters,[29,30] and (3) capsid-mutagenesis of AAV vectors.[31–35]

Recently, human serum albumin (HSA) was shown to increase the transduction efficiency of all AAV serotype vectors,[36] and more recently, polyvinyl alcohol (PVA), was reported to be a superior replacement for HSA for *ex vivo* expansion of HSCs.[37] In the present studies, we evaluated whether PVA could also enhance the transduction efficiency of AAV6 vectors in primary human HSCs. We provide experimental evidence that PVA can increase the transduction efficiency of AAV6 vectors in primary human HSCs up to 12-fold, which is mediated through improved entry and intracellular trafficking of AAV6 vectors, both *in vitro* and *in vivo*. This has implications in the optimal use of these vectors in the potential gene therapy and genome editing for human hemoglobinopathies.

RESULTS

PVA Improves the Transduction Efficiency of AAV6 Vectors in Human Hematopoietic Cells

As stated above, since HSA has been shown to improve the transduction efficiency of AAV vectors,[36] and since PVA was recently shown to be a superior replacement for HSA to dramatically promote *ex vivo* expansion of murine HSCs,[37] we wished to evaluate the effect of PVA on the transduction efficiency of AAV6 vectors in primary human HSCs. PVA is a synthetic polymer derived from polyvinyl acetate by hydrolysis. Typical levels of hydrolysis range from 80% to greater than 99%.[38] We used both 87% hydrolyzed PVA (PVA87), and more than 99% hydrolyzed PVA (PVA99) in our preliminary experiments using K562 cells, frequently used as a model for hematopoietic cell transduction studies. AAV6 vectors expressing the enhanced green fluorescence protein (EGFP) reporter gene under the control of a cytomegalovirus (CMV) enhancer-chicken β-actin promoter (CBA) were either mock-treated or pre-incubated with PVA concentration ranging from 0.001% to 1% and used to transduce K562 cells in triplicates under identical conditions. Transduction efficiency was evaluated by EGFP expression 48 h post-transduction using flow cytometry. These results are shown in Figure 1. As can be seen, whereas low concentration of PVA had no effect, a significant increase in the transduction efficiency of AAV6 vectors was observed with preincubation with 1% concentration of both PVA87 (Figure 1A) and PVA99 (Figure 1B). Since PVA is known to be non-cytotoxic, no apparent cytotoxicity in K562 cells was observed (Figure S1). Since the extent of the increase in transgene expression with PVA87 was more pronounced

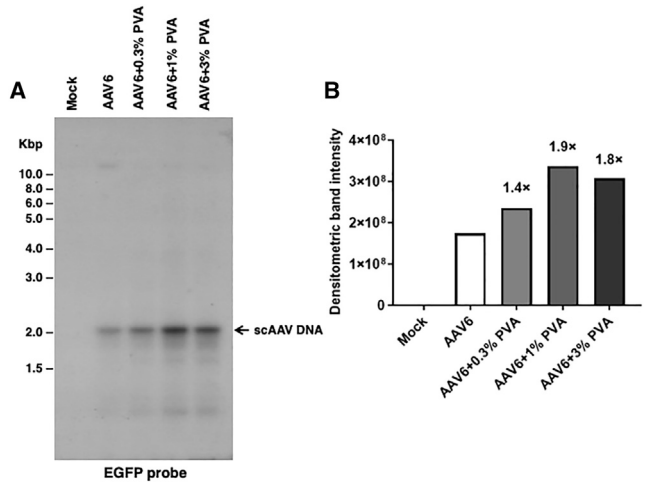

Figure 3. PVA Improves AAV6 Vector Uptake in K562 Cells *In Vitro*

K562 cells were transduced with 1 × 10^3 vgs/cell of scAAV-CBAp-EGFP vectors, with or without pre-incubation with various indicated amounts of PVA87. Low molecular weight DNA samples were isolated 2 h post-transductions and analyzed on a Southern blot using ^{32}P-labeled EGFP-specific DNA probe (A). Quantitation of the data using the ImageX software (B).

than that with PVA99, all subsequent studies were carried out with PVA87.

PVA-AAV6 Vector Interaction Is Critical for the Increased Transduction Efficiency

In the next set of experiments, we evaluated whether the transduction efficiency of AAV6 vectors could be further increased with higher concentration of PVA87. AAV6-CBA-EGFP vectors were preincubated with PVA87 at concentrations ranging from 0.03% to 3%, and transgene expression was determined as described above. These results, shown in Figure 2A, document that a dose-dependent increase, up to 9-fold, in transgene expression was achieved with pre-incubation of AAV6 vectors with 3% PVA87. It was next of interest to determine which step in the AAV6 vector life cycle was influenced by PVA87. To this end, the following sets of experiments were performed: (1) no treatment; (2) pre-incubation of AAV6 vectors with 1% PVA87; (3) pre-incubation of K562 cells with 1% PVA87 followed by AAV6 vector transduction; (4) addition of 1% PVA87 prior to transduction with AAV6 vectors; and (5) addition of 1% PVA87 following transduction with AAV6 vectors. Transgene expression was determined as described above. These results are shown in Figure 2B. It is evident that the extent of the increase in the transduction efficiency was most pronounced when PVA87 was pre-incubated with the vector, suggesting that a direct interaction between AAV6 and PVA87 is a critical step for the observed increase in the transduction efficiency, with no apparent cytotoxicity (Figure S2).

PVA Enhances AAV6 Vector Uptake in Human Hematopoietic Cells

Since a direct interaction between AAV6 and PVA87 appeared to be critical, we hypothesized that the observed increased transduction was a consequence of enhanced AAV6 vector uptake into cells. This possibility was tested experimentally by determining the extent of the vector entry in the absence or the presence of increasing amounts of PVA87, followed by detection of intracellular vector genome by Southern blot analysis. These results are shown in Figures 3A. As can be seen, pre-incubation with PVA87 led to increased uptake of AAV vector genomes in K562 cells.

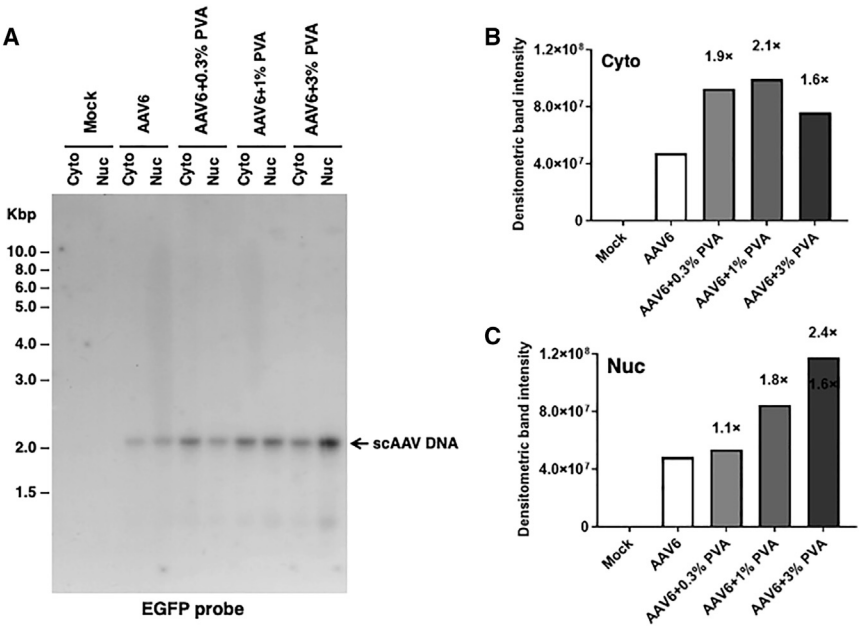

Figure 4. PVA Improves Nuclear Transport of AAV6 Vectors in K562 Cells *In Vitro*

Southern blot analysis was performed with low mol. DNA samples isolated from cytoplasmic and nuclear fractions from K562 cells transduced with 3 × 10^3 vgs/cell of scAAV6-CBAp-EGFP vectors, with or without pre-incubation with various concentrations of PVA87, 2 h post-transductions, and analyzed on a Southern blot and probed with the EGFP-specific probed as described above (A). Densitometric scanning of the Southern blot and quantitation of the data were performed using the ImageX software for cytoplasmic (Cyto) (B), and nuclear (Nuc) (C) fractions.

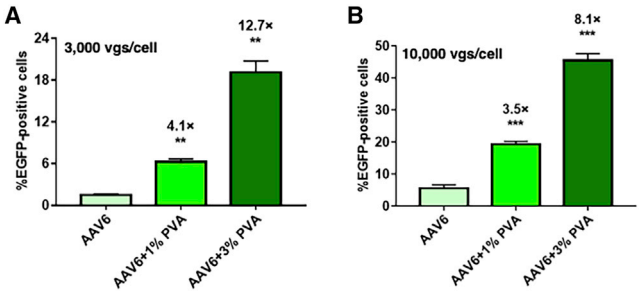

Figure 5. PVA Increases the Transduction Efficiency of AAV6 Vectors in Primary Human HSCs *In Vitro*

(A and B) Primary human bone-marrow-derived $CD34^+$ cells were transduced with scAAV6-CBAp-EGFP vectors at 3×10^3 (A) and 1×10^4 (B) vgs/cell, respectively, with or without pre-incubation with 1% or 3% PVA87 at 4°C for 2 h. Transgene expression was determined by flow cytometry 48 h post-transduction. Statistical significances are indicated as $^{**}p < 0.01$, $^{***}p < 0.001$.

PVA Enhances Nuclear Transport of AAV6 Vectors in Human Hematopoietic Cells

Since increased intracellular vector uptake alone could not account for increased transduction, unless the vectors also trafficked to the nucleus, in the next set of experiments, we further analyzed the distribution of vector genomes in the cytoplasmic and the nuclear fractions. To this end, AAV6 vectors, with or without pre-incubation with increasing amounts of PVA87, were used to transduce K562 cells. Nuclear and cytoplasmic fractions were analyzed for the presence of vector genomes by Southern blot analysis as described above. These results, shown in Figure 4, clearly indicate that pre-incubation with PVA87 also led to increased nuclear transport of AAV vector genomes into K562 cells and appeared to correlate well with the PVA87 concentration used.

PVA Increases the Transduction Efficiency of AAV6 Vectors in Primary Human HSCs *In Vitro*

To further evaluate whether PVA87 could also increase the transduction efficiency of AAV6 vectors in HSCs, we transduced primary human bone-marrow-derived $CD34^+$ cells with self-complementary AAV6 (scAAV6)-CBAp-vectors at 3,000 and 10,000 vgs/cell, with or without pre-incubation with 1% or 3% PVA87, and analyzed them for transgene expression as described for K562 cells. These results are shown in Figure 5. As can be seen, at 3,000 vgs/cell, up to 12-fold enhancement of transduction efficiency was observed with 3% PVA87, whereas the transduction efficiency was enhanced up to 8-fold with 3% PVA87 at 10,000 vgs/cell, with no apparent cytotoxicity (Figure S3).

PVA Enhances the Transduction Efficiency of AAV6 Vectors in Murine Hepatocytes *In Vivo* with No Apparent Hepato-toxicity

We also wished to examine whether PVA87 could augment the transduction efficiency of AAV6 vectors in an animal model *in vivo*. Because AAV6 vectors do not transduce primary murine HSCs,[21] we evaluated the transduction efficiency of AAV6 vectors in mouse liver following intravenous delivery. Three groups (n = 5 each) of normal C57BL/6 mice were administered via the tail-vein with 1×10^{10} vgs of ssAAV6 vectors expressing the firefly luciferase (FLuc) reporter gene under the control of the CBA promoter, with or without pre-incubation with 1% or 3% PVA87. Two weeks post-vector administrations, whole-body bioluminescence imaging was performed and quantitated. These results are shown in Figures 6A and 6B. It is interesting to note that pre-incubation with 1% PVA87 led to 4-fold increase, and with 3% PVA87 led to 7-fold fold increase in AAV6 transduction efficiency in murine hepatocytes *in vivo*. These results suggested, but did not prove, that the observed PVA87-mediated increased efficiency was also due to increased intracellular trafficking of AAV6 vectors in murine hepatocytes. Vector genome copy numbers were determined in DNA samples from liver tissues from mice from each of the three groups using quantitative polymerase chain reaction (qPCR) with FLuc-specific primers. These results, shown in Figure 6C, nonetheless, corroborated that the PVA87-mediated improved transduction efficiency correlated with increased vector uptake and entry into mouse hepatocytes *in vivo*. The potential PVA-induced liver toxicity was examined by determining the blood levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which are indicative of liver damage. 2 weeks post-vector administration, both AST and ALT levels in mice from each of the three groups were observed to be within the normal range (data not shown), indicating that PVA-treatment is not associated with liver toxicity at the concentration tested.

DISCUSSION

Since our first identification of AAV6 as the most efficient serotype vector for transduction of primary human HSCs,[21] several other independent groups have not only corroborated these observations, but further documented that AAV6 vectors are also highly efficient in mediating genome editing in primary human HSCs, as well as in primary human T cells.[22–24] However, relatively high doses, ranging from 100,000–200,000 vgs/cell are required to achieve 25%–55% efficacy, including genome editing of the sickle mutation with the CRISPR/Cas9 system.[25] In order to reduce, if not completely eliminate, the possibility of off-target cleavage, it would be desirable to limit the vector dose to as low a level as possible.

We have reported the development of capsid-modified AAV6 vectors with which transduction efficiency exceeding 90% can be achieved in primary human HSCs at an MOI of 20,000 vgs/cell.[39,40] Thus, it is conceivable that PVA could be safely used to further reduce the AAV6 vector dose to achieve safe and efficient genome editing in HSCs from patients with β-thalassemia and sickle cell disease, especially since PVA has been widely used in a number of medical devices, due to its biocompatibility, low toxicity, and low protein adsorption characteristics,[38] and in our studies, PVA was not found to induce liver toxicity in mice. In this context, however, it is also important to note that although AAV6 vectors efficiently deliver donor DNA templates in conjunction with nuclease-based editing platforms such as zinc finger nucleases and CRISPR/Cas9,[22–25] the AAV6-delivered donors are recombined at the sites of

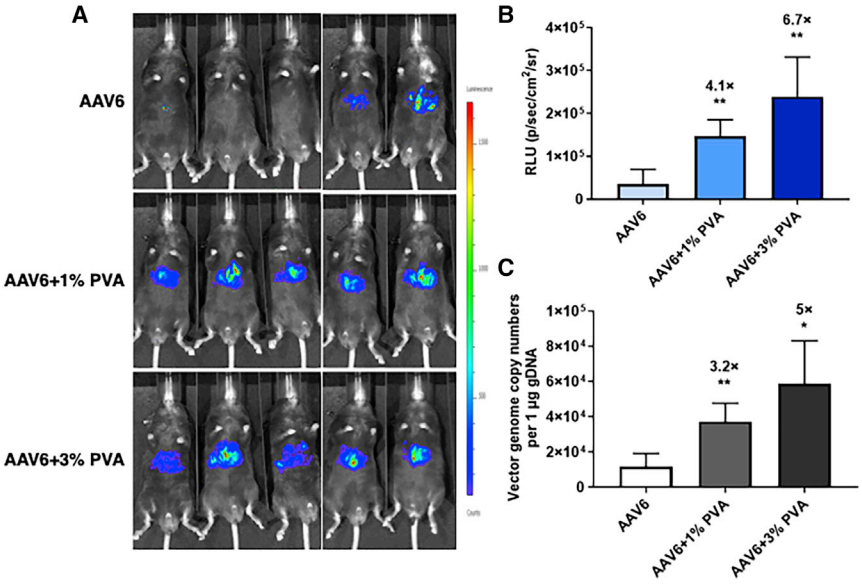

Figure 6. PVA Augments the Transduction Efficiency of AAV6 Vectors in Mouse Liver *In Vivo*

ssAAV6-CBAp-FLuc vectors, with or without pre-incubation with 1% or 3% PVA87 at 4°C for 2 h, were injected via tail vein in C57BL/6 mice at 1×10^{10} vgs/mouse. Whole-body bioluminescence images were acquired 2 weeks post-vector administration (A). Quantitation of bioluminescence signal intensity is shown as photons/second/cm^2/steradian (p/sec/cm^2/sr) (B). AAV6 vector genome copy numbers in mouse liver were quantified by qPCR using FLuc-specific primers (C). Statistical differences are indicated as $^*p < 0.05$, $^{**}p < 0.01$. RLU, relative light units; gDNA, genomic DNA.

double-stranded DNA breaks induced by nucleases such as CRISPR, and that AAV6 alone does not induce genome editing in the absence of nuclease treatment.[41–43]

More recently, novel human stem-cell-derived clade F AAV vectors were shown to mediate high-efficiency homologous recombination-based genome editing in primary human HSCs.[44] Although it has been claimed that AAV6 is superior to clade F AAV vectors in mediating homologous recombination-based genome editing in human HSCs,[45] and that homologous recombination-based genome editing by clade F AAV vectors is inefficient in the absence of a targeted DNA break,[46] the underlying reason for these apparent discrepancies has been explained by the requirement of exceedingly high MOIs, 150,000 vgs/cell or higher, to achieve nuclease-free homologous recombination by clade F AAV vectors.[47] Thus, it would be of interest to evaluate whether PVA-treatment can augment the genome editing efficiency of AAVHSC vectors as well, thereby significantly reducing the need to use high MOIs.

In our current study, we observed that PVA, which was recently reported to be a superior replacement for HSA in a dramatic *ex vivo* expansion of murine HSCs,[37] could also significantly improve the transduction efficiency of AAV6 vectors in primary human HSCs. Whether PVA can also mediate *ex vivo* expansion of primary human HSCs remains to be documented. In this context, it is noteworthy that significant expansion of primary human HSCs in a zwitterionic hydrogel in a 3D culture was recently reported.[48] What effect, if any, these conditions have on the transduction efficiency of AAV6 vectors remains to be investigated. Although further studies are warranted to gain a better understanding of the underlying mechanism of AAV6-PVA interactions, we were able to demonstrate that the improvement in the transduction efficiency was due to PVA-mediated improved entry and intracellular trafficking of AAV6 vectors in human hematopoietic cells *in vitro*, as well as in murine hepatocytes *in vivo*.

Whether the observed effect of PVA-mediated enhancement of transduction is specific to AAV6 serotype vectors, or the transduction efficiencies of other clinically relevant AAV serotype vectors, such as AAV3, AAV5, AAV8, and AAV9, can be similarly augmented by PVA, remains to be examined. In this context, however, it is noteworthy that PVA failed to increase the transduction efficiency of AAV2 serotype vectors in several human cell lines *in vitro* (H.Y., K.Q., M.T., W.W., A.S., unpublished data). Further studies are currently underway to address some of the unanswered questions outlined above.

MATERIALS AND METHODS

Cell Lines, Primary Cells, Cell Cultures, and Reagents

Human embryonic kidney 293 (HEK293) and erythroleukemia K562 cells were purchased from American Type Culture Collections (ATCC, Manassas, VA, USA) and maintained at 37°C in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM; Lonza, Walkersville, MD, USA) supplemented with 10% fetal bovine serum (FBS; Sigma, St. Louis, MO, USA) and 1% penicillin-streptomycin (Invitrogen, Grand Island, NY, USA). Human bone marrow CD34$^+$ cells were purchased from AllCells (AllCells Technologies, Emeryville, CA, USA) and maintained at 37°C in 5% CO_2 in StemSpan Serum-Free Expansion Medium (SFEM; StemCell Technologies, Vancouver, BC, Canada) with StemSpan CC100 (StemCell Technologies, Vancouver, BC, Canada). PVA87 (87%–90% hydrolyzed, average molecular weight 30,000–70,000; catalog no. P8136) and PVA99 (99+% hydrolyzed, average molecular weight 85,000–124,000; catalog no. 363146) were purchased from Sigma-Aldrich, St. Louis, MO, USA.

Viral Vector Production

The scAAV plasmid containing the CBAp-EGFP transgene expression cassette has been described previously.[49] scAAV6-CBAp-EGFP vector was packaged using the triple-plasmid transfection method, mediated by polyethyleneimine[50] (PEI, linear, MW 25000; Polysciences, Warrington, PA, USA). HEK293 cells were

harvested 72 h post-transfection, and lysed by 3 rounds of freeze-thaw, and digested with Benzonase (Invitrogen, Grand Island, NY, USA). Cell debris was removed by centrifugation. AAV6 vectors were purified by iodixanol (Sigma, St. Louis, MO, USA) gradient ultracentrifugation, followed by ion exchange chromatography using HiTrap SP/Q HP columns (GE Healthcare, Piscataway, NJ, USA), washed with PBS and concentrated by centrifugation using centrifugal spin concentrators with 150 kDa molecular weight cut-off. Titers were determined by quantitative real-time PCR assays as previously described. rAAV6-CBAp-FLuc was purchased from PackGene (PackGene Biotech, Worcester, MA, USA).

AAV Transduction Assay *In Vitro*

Human K562 cells (1×10^5) and primary human $CD34^+$ cells (5×10^4) were seeded in Falcon round bottom polystyrene tubes (Corning, NY, USA). Cells were resuspended in DMEM before transduction. scAAV6 vectors expressing the EGFP reporter gene under the control of a cytomegalovirus (CMV) enhancer-CBA were either mock-treated or pre-incubated with PVA concentration ranging from 0.001% to 3% and used to transduce cells in triplicates under identical conditions. DMEM was replaced by culture medium 2 h post-transduction. EGFP expression was determined 48 h post-transduction using flow cytometry (Accuri C6, Beckton Dickinson, Franklin Lakes, NJ, USA), followed by processing with software FCS Express 6 Flow.

Southern Blot Analyses

Low molecular weight DNA samples isolated from whole-cells or nuclear and cytoplasmic fractions from K562 cells were isolated by the method described by Hirt.[51] DNA fragments were electrophoresed on 1% agarose gels, transferred to nylon membranes, hybridized with ^{32}P-labeled EGFP DNA probe and subjected to autoradiography. Densitometric scanning of autoradiograms was performed with a Digital Imaging System Alphaimager (Alpha Innotech, San Leandro, CA, USA).

Animal Experiments

Male C57BL/6 mice were purchased from the Jackson Laboratory and maintained in the University of Florida Animal Care Facility. All experimental protocols involving animals were approved by the Institutional Animal Care and Use Committee guidelines. 1×10^{10} particles of ssAAV6-CBAp-FLuc vectors were incubated with or without PVA, in a total volume of 200 μL, at 4°C for 2 h, followed by tail-vein injections (n = 5 per group). 2 weeks post-vector injections, whole-body bioluminescence imaging was performed using a Xenogen IVIS Lumina imaging system (Caliper Lifesciences, Hopkinton, MA, USA) following intraperitoneal injection of luciferin substrate (Nanolight, Pinetop, AZ, USA) at 150 mg per kg of mouse body weight. Bioluminescence images were analyzed using Living Image software (Caliper Lifesciences, Hopkinton, MA, USA). AAV6 vector genome copy numbers were determined by qPCR using total genomic DNA isolated from liver tissues.

Statistical Analysis

Quantitative results were expressed as means ± SD. Unpaired Student's t test was performed for statistical analysis. p values < 0.05 were considered as statistically significant. Statistical significances are indicated as *p < 0.05, **p < 0.01, ***p < 0.001.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.omtn.2020.03.009.

AUTHOR CONTRIBUTIONS

H.Y., K.Q., G.D.K., L.Y., and M.M. designed and performed the experiments. H.Y., K.Q., G.D.K., L.Y., M.M., C.L., B.E.H., M.A.-M., M.T., W.W., and A.S. analyzed the data. K.Q. and A.S. conceived of the idea. H.Y. and A.S. wrote the manuscript, and all authors read and approved the final version.

CONFLICTS OF INTEREST

A.S. is a cofounder of, and holds equity in, Lacerta Therapeutics and Nirvana Therapeutics and is an inventor on several issued patents on recombinant AAV vectors that have been licensed to various gene therapy companies. M.A.-M. is a scientific advisor of Voyager Therapeutics, a member of the Scientific Advisory Board (SAB) for Applied Genetic Technologies Corporation (AGTC), is a consultant for Intima Biosciences, and has a sponsored research agreement with Intima Bioscience and Voyager Therapeutics. M.A.-M. is also a co-founder of StrideBio. M.A.-M. and M.M. have intellectual property (IP) on AAV vectors. All other authors declare no competing interests.

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