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Human Hepatocyte Growth Factor Receptor Is a Cellular Coreceptor for Adeno-Associated Virus Serotype 3

Chen Ling,[1–3] Yuan Lu,[1,3] Jasmine K. Kalsi,[1,3] Giridhara R. Jayandharan,[1,3–5] Baozheng Li,[1,3] Wenqin Ma,[1,3] Binbin Cheng,[1,3] Samantha W.Y. Gee,[1,3,6] Katherine E. McGoogan,[1,3,7] Lakshmanan Govindasamy,[8] Li Zhong,[9] Mavis Agbandje-McKenna,[3,8] and Arun Srivastava[1,3,10,11]

Abstract

Adeno-associated viruses (AAVs) use a variety of cellular receptors/coreceptors to gain entry into cells. A number of AAV serotypes are now available, and the cognate receptors/coreceptors for only a handful of those have been identified thus far. Of the 10 commonly used AAV serotypes, AAV3 is by far the least efficient in transducing cells in general. However, in our more recent studies, we observed that AAV3 vectors transduced human liver cancer cells remarkably well, which led to the hypothesis that AAV3 uses hepatocyte growth factor receptor (HGFR) as a cellular coreceptor for viral entry. AAV3 infection of human liver cancer cell lines was strongly inhibited by hepatocyte growth factor, HGFR-specific small interfering RNA, and anti-HGFR antibody, which corroborated this hypothesis. However, AAV3 vectors failed to transduce murine hepatocytes, both *in vitro* and *in vivo*, suggesting that AAV3 specifically uses human HGFR, but not murine HGFR, as a cellular coreceptor for transduction. AAV3 may prove to be a useful vector for targeting human liver cancers for the potential gene therapy.

Introduction

Recombinant vectors based on a nonpathogenic human parvovirus, adeno-associated virus 2 (AAV2), have been developed and are currently in use in a number of gene therapy clinical trials (Daya and Berns, 2008). More recently, a number of additional AAV serotypes have also been isolated (Muramatsu *et al.*, 1996; Chiorini *et al.*, 1997, 1999; Rutledge *et al.*, 1998; Gao *et al.*, 2002, 2004), which have been shown to exhibit selective tissue tropism in various small and large animal models (Zincarelli *et al.*, 2008). Whereas a number of cellular receptors/coreceptors have been identified for AAV2 (Summerford and Samulski, 1998; Qing *et al.*, 1999; Summerford *et al.*, 1999; Kashiwakura *et al.*, 2005; Asokan *et al.*, 2006), only a handful of receptors/coreceptors for other AAV serotypes have been described (Kaludov *et al.*, 2001; Walters *et al.*, 2001; Di Pasquale *et al.*, 2003; Akache *et al.*, 2006; Wu *et al.*, 2006).

We reported that of the 10 commonly used serotypes, AAV3 vectors were by far the most efficient in transducing established human hepatoblastoma (HB) and human hepatocellular carcinoma (HCC) cell lines as well as primary human hepatocytes (Glushakova *et al.*, 2009). Although transduction by AAV3 serotype vectors has been reported to be inhibited by heparin, heparan sulfate, and fibroblast growth factor receptor-1 (FGFR1) (Rabinowitz *et al.*, 2004; Blackburn *et al.*, 2006), the true identity of a cellular receptor/coreceptor remains unclear. Because HB and HCC cells express elevated levels of hepatocyte growth factor receptor (HGFR) (Grigioni *et al.*, 1995; Okano *et al.*, 1999), we hypothesized that AAV3 uses HGFR as a possible receptor/coreceptor for entry into liver-specific cells.

[1]Division of Cellular and Molecular Therapy, Department of Pediatrics, University of Florida College of Medicine, Gainesville, FL 32611.
[2]Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, FL 32610.
[3]Powell Gene Therapy Center, University of Florida College of Medicine, Gainesville, FL 32610.
[4]Department of Haematology, Christian Medical College, Vellore, Tamil Nadu 632004, India.
[5]Centre for Stem Cell Research, Christian Medical College, Vellore, Tamil Nadu 632002, India.
[6]Division of Critical Care Medicine, Department of Pediatrics, University of Florida College of Medicine, Gainesville, FL 32610.
[7]Division of Gastroenterology, Department of Pediatrics, University of Florida College of Medicine, Gainesville, FL 32610.
[8]Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, FL 32610.
[9]Gene Therapy Center and Division of Hematology/Oncology, Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01605.
[10]Genetics Institute, University of Florida College of Medicine, Gainesville, FL 32611.
[11]Shands Cancer Center, University of Florida College of Medicine, Gainesville, FL 32611.

In this brief report, using a variety of methods, we document that interference with the cell surface presence or expression of HGFR significantly reduces the transduction efficiency of AAV3 vectors. Furthermore, both *in vitro* and *in vivo* data suggest that AAV3 specifically uses human HGFR (hHGFR), and not mouse HGFR (mHGFR), as a cellular coreceptor to gain entry into cells.

Materials and Methods

Cell lines and cultures

Human cervical cancer (HeLa) and hepatocellular carcinoma (Huh7), and murine adult hepatocyte (H2.35) cell lines were purchased from the American Type Culture Collection (Manassas, VA) and maintained in complete Dulbecco's modified Eagle's medium (DMEM; Mediatech, Manassas, VA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO) and 1% penicillin and streptomycin (P/S; Lonza, Walkersville, MD). A newly established human hepatoblastoma (Hep293TT) cell line was generously provided by G.E. Tomlinson (University of Texas Health Science Center at San Antonio, San Antonio, TX) and was maintained in complete RPMI medium 1640 (Invitrogen, Carlsbad, CA) supplemented with 15% heat-inactivated FBS (Sigma-Aldrich) and 1% P/S (Lonza). Cells were grown as adherent cultures in a humidified atmosphere at 37°C in 5% CO_2 and were subcultured after treatment with trypsin–Versene mixture (Lonza) for 2–5 min at room temperature, washed, and resuspended in complete medium.

Recombinant AAV vectors

Highly purified stocks of self-complementary AAV2 (scAAV2) and scAAV3 vectors carrying the enhanced green fluorescence protein (EGFP) gene driven by the chicken β-actin promoter were packaged by the calcium phosphate triple-plasmid transfection protocol described previously (Auricchio *et al.*, 2001; Wu *et al.*, 2007). Physical particle titers of recombinant vector stocks were determined by quantitative DNA slot-blot analyses (Kube and Srivastava, 1997).

AAV vector transduction in vitro

Huh7 or HeLa cells were seeded in 96-well plates at a concentration of 5000 cells per well in complete DMEM. AAV infections were performed in serum- and antibiotic-free DMEM. Hep293TT cells were seeded in 96-well plates at a concentration of 10,000 cells per well in complete RPMI medium. Infections were performed in serum- and antibiotic-free RPMI medium. Expression of EGFP was analyzed either by flow cytometry or by direct fluorescence imaging 72 hr after transduction.

Animal handling

All animal experiments were performed according to the guidelines for animal care specified by the Animal Care Services at the University of Florida (Gainesville, FL). Ten-week-old C57BL/6J male mice were purchased from Jackson Laboratory (Bar Harbor, ME) and maintained at the University of Florida College of Medicine (Gainesville, FL). The Institutional Animal Care and Use Committee approved all protocols for the care and use of these mice.

Recombinant AAV vector transduction in vivo

scAAV-EGFP vectors were injected intravenously via the tail vein into C57BL/6 mice at 1×10^{10} vector genomes (VG) per animal. Phosphate-buffered saline (PBS)-injected mice were used as an appropriate control. Livers were harvested from mice 8 weeks after vector administration, and thin sections from each hepatic lobes were mounted on slides and visualized by fluorescence microscopy. Four representative sections of each lobe were examined to determine transduction efficiency, using National Institutes of Health (NIH, Bethesda, MD) ImageJ software.

Statistical analysis

Results are presented as means $\pm$ standard deviation (SD). Differences between groups were identified by Student *t* test, using a grouped-unpaired two-tailed distribution. $p < 0.05$ was considered statistically significant.

Results

AAV3 infection is strongly inhibited by HGF

To test our hypothesis that AAV3 uses hepatocyte growth factor receptor (HGFR) as a putative cellular coreceptor, we evaluated the effect of hepatocyte growth factor (HGF), the ligand for HGFR, on AAV3 transduction. Huh7, a human hepatocellular carcinoma (HCC) cell line, was transduced with self-complementary (sc) AAV3-EGFP vectors in the absence or presence of increasing concentrations of HGF (Invitrogen) at 37°C for 2 hr and then rinsed twice with PBS. Transgene expression was analyzed by flow cytometry 72 hr posttransduction. These results, shown in Fig. 1A, indicate that HGF reduced scAAV3 vector-mediated transgene expression in a dose-dependent manner. HGF concentration as low as 5 μg/ml resulted in greater than 85% inhibition. Next, to rule out any possible nonspecific nature of this inhibition, preincubation and postincubation studies were performed as follows. Huh7 cells were first incubated with HGF (5 μg/ml) at 37°C for 2 hr, washed extensively, and then infected with scAAV3-EGFP vectors, or cells were first infected with scAAV3-EGFP vectors, washed extensively, and then incubated with HGF (5 μg/ml) at 37°C for 2 hr. scAAV2-EGFP vectors were also included as appropriate controls because HGFR has previously been shown to be a coreceptor for AAV2 (Kashiwakura *et al.*, 2005). These results, shown in Fig. 1B, indicate that AAV3 vectors transduce Huh7 cells more efficiently than AAV2 vectors, which is consistent with our previously published studies (Glushakova *et al.*, 2009). Whereas preincubation with HGF led to significant inhibition of transduction by both AAV2 and AAV3 vectors, postincubation with HGF had no significant effect. These data suggest that AAV3 interacts directly with HGFR, and that HGF inhibits an early event in viral infection.

In addition to Huh7 cells, which were established nearly 30 years ago (Nakabayashi *et al.*, 1982), we wished to investigate whether AAV3 vectors could also transduce human liver cancer cells that were established more recently from primary tumor tissue, to rule out any tissue culture artifact. For this purpose, Hep293TT, a recently established cell line resected from a child with an aggressive hepatoblastoma (HB) (Chen *et al.*, 2009; Glushakova *et al.*, 2009), was transduced with scAAV3-EGFP vectors in the absence or presence

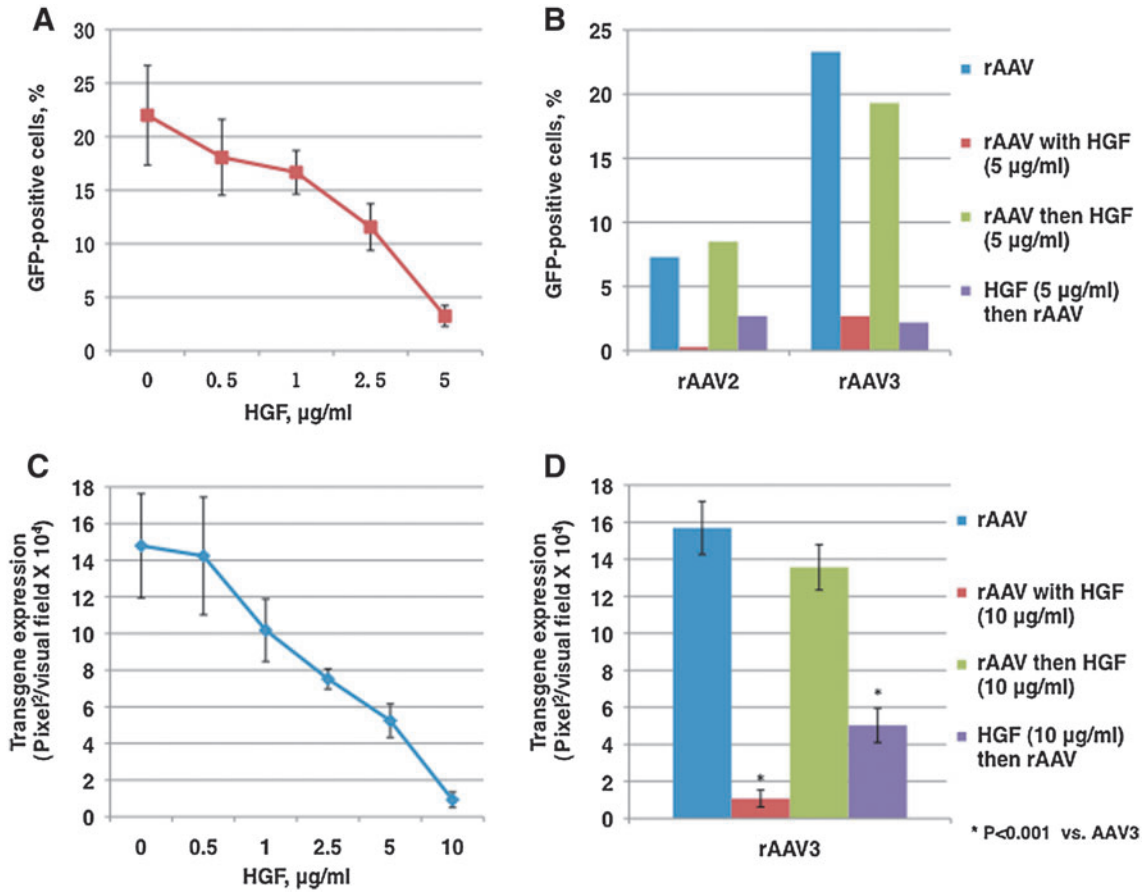

FIG. 1. (**A**) AAV3 vector-mediated transgene expression in Huh7 cells in the presence of various concentrations of HGF. Cells were transduced with vector at 1×10^4 VG/cell, and EGFP-positive cells were enumerated 72 hr posttransduction. (**B**) Effect of pre- and postincubation of Huh7 cells with HGF (5 μg/ml) on the transduction efficiency of AAV2 and AAV3 serotype vectors. (**C**) AAV3 vector-mediated transgene expression in Hep293TT cells in the presence of various concentrations of HGF. Cells were transduced with vector at 2×10^3 VG/cell, and EGFP-positive cells were enumerated 72 hr posttransduction. (**D**) Effect of pre- and postincubation of Hep293TT cells with HGF (10 μg/ml) on the transduction efficiency of and AAV3 serotype vectors.

of increasing concentrations of HGF as described previously. Seventy-two hours postinfection, the transduction efficiency of AAV3 was measured by GFP imaging, using a fluorescence microscope (DMI 4000E; Leica Microsystems, Wetzlar, Germany). Images from three wells of vector-infected cells were analyzed quantitatively with ImageJ analysis software (NIH). Transgene expression was assessed as total area of green fluorescence (pixel^2) per visual field. These results, shown in Fig. 1C, indicate that AAV3 vectors transduced Hep293TT as efficiently as Huh7 cells, and that this transduction was strongly inhibited by coincubation with HGF. Consistent with results obtained with Huh7 cells, incubation of Hep293TT cells with HGF postinfection by AAV3 vectors had little effect on transduction efficiency (Fig. 1D).

HGFR small interfering RNA and HGFR antibody also strongly inhibit transduction by AAV3 vectors

We next used HGFR-specific small interfering RNA (siRNA) to knock down HGFR expression, and evaluated the impact on AAV3 vector-mediated transduction in Huh7 cells. The expression of HGFR after transfection with 10 pmol of negative control siRNA or HGFR-specific siRNA (Invitrogen) was analyzed by fluorescence-activated cell sorting (FACS) using anti-human HGFR antibody, which indicated that HGFR expression was reduced from 89.6 to 7.5% (data not shown). Negative control siRNA- and HGFR-specific siRNA-transfected Huh7 cells were transduced with scAAV3-EGFP vectors as described previously. Transgene expression was analyzed by flow cytometry 72 hr posttransduction. The transduction efficiency of AAV3 vectors was decreased from ~23% in negative control siRNA-transfected cells to ~8% in HGFR-specific siRNA-transfected cells (Fig. 2A). AAV3 vector-mediated transduction of Huh7 cells was also strongly inhibited by coincubation of cells with a 100-μg/ml concentration of anti-HGFR polyclonal antibodies (R&D Systems, Minneapolis, MN), specific for the extracellular domain of hHGFR (Fig. 2B).

AAV3 vectors transduce murine hepatocytes inefficiently, both in vitro *and* in vivo

We next wished to examine whether AAV3 uses murine HGFR as a coreceptor in an animal model *in vivo*, especially because HGFR has been shown to be a coreceptor for AAV2 (Kashiwakura *et al.*, 2005), and because transduction by both

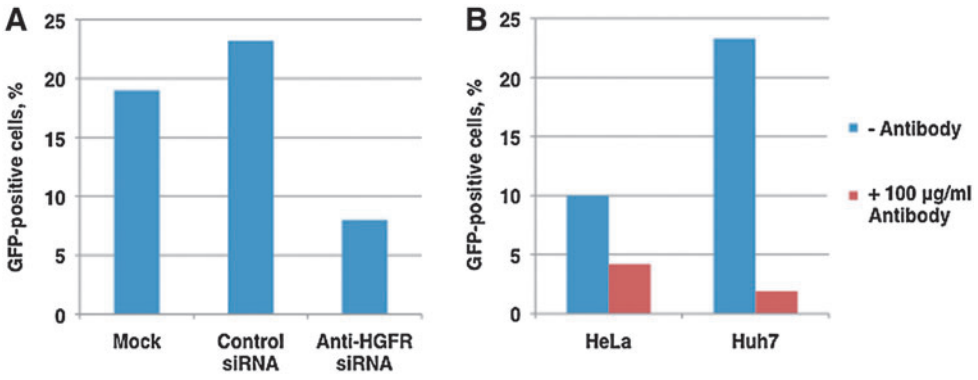

FIG. 2. (**A**) Effect of transfection with 10 pmol of either negative control siRNA or HGFR-specific siRNA on AAV3 vector-mediated transduction of Huh7 cells. (**B**) AAV3 vector-mediated transgene expression in Huh7 cells in the absence or presence (100 μg/ml) of anti-HGFR antibody. Transgene expression was determined 72 hr posttransduction.

AAV2 and AAV3 serotype vectors has been shown to be inhibited by heparin, implying that heparan sulfate proteoglycan (HSPG) serves as a cellular receptor for both AAV2 and AAV3 (Rabinowitz *et al.*, 2004; Blackburn *et al.*, 2006). To this end, $\sim 1 \times 10^{10}$ VG of scAAV3-EGFP vector were injected via the tail vein into male C57BL6/J mice, and transgene expression was evaluated in liver tissues 8 weeks postinjection. scAAV2-EGFP and scAAV8-EGFP vectors, known to transduce murine hepatocytes at low and high efficiencies, respectively (Gao *et al.*, 2002; Thomas *et al.*, 2004), were included as appropriate controls. These results, shown in Fig. 3A, clearly indicate that whereas efficient transduction of primary murine hepatocytes occurred with AAV8, followed by AAV2 vectors, transduction by AAV3 vectors was largely inefficient. In *in vitro* experiments, AAV2 vectors transduced either Hep293TT or H2.35, a mouse adult hepatocyte cell line, at the same efficiency. However, the efficiency of AAV3 vector-mediated transduction of H2.35 cells was reduced by several orders of magnitude compared with that of Hep293TT (data not shown).

Discussion

Ever since the realization that infection by AAV serotype 2 (AAV2) is receptor mediated (Ponnazhagan *et al.*, 1996), concerted efforts have been made to identify the cellular receptor for AAV2, and heparan sulfate proteoglycan (HSPG) was documented to be the primary receptor for AAV2 (Summerford and Samulski, 1998). Further studies revealed that HSPG alone is insufficient, and that AAV2 also requires cellular coreceptors that mediate a successful infection (Qing *et al.*, 1999; Summerford *et al.*, 1999). To date, at least four additional cellular coreceptors have been identified for AAV2, including HGFR (Walters *et al.*, 2001; Kashiwakura *et al.*, 2005; Akache *et al.*, 2006; Wu *et al.*, 2006; Kurzeder *et al.*, 2007). This is of interest because additional AAV serotypes have also been reported to use cellular growth factor receptors as coreceptors (Di Pasquale *et al.*, 2003; Blackburn *et al.*, 2006; Weller *et al.*, 2010).

However, because the transduction efficiency of AAV3 vectors in general has been reported to be particularly low, both *in vitro* and *in vivo*, our observation that these vectors transduce human liver cancer cell lines exceedingly well (Glushakova *et al.*, 2009) prompted us to hypothesize that AAV3 uses human HGFR as a putative cellular coreceptor. From the data presented here, we surmise that AAV3 does not use murine HGFR (mHGFR) as a coreceptor, despite 88% shared identity between hHGFR and mHGFR, most likely because the variations are localized in the extracellular domains, the most probable virus interaction site, and because mHGF has been shown to be an ineffective ligand for hHGFR (Rong *et al.*, 1992; Okano *et al.*, 1999). Indeed, mapping of mHGFR and hHGFR amino acid differences onto the crystal structure of the extracellular domain of hHGFR in complex with the α domain of HGF (Stamos *et al.*, 2004), depicted in Fig. 3B, shows the distribution of the differing residues on the outer surface of hHGFR and a clustering at the interaction interface. The location of amino acid differences along the interaction interface, including residues observed to contact HGF, may account for the lack of use of mHGFR as a cellular coreceptor by AAV3, if a similar HGFR interaction site is used by the virus. Additional studies on AAV3–hHGFR interactions, using the labeled virus, transfection experiments using a human HGFR expression plasmid in a murine liver cancer cell line *in vitro*, and hydrodynamic injection of a human HGFR plasmid in a mouse model *in vivo*, beyond the scope of the current study, will further corroborate the current observation that AAV3 preferentially uses human HGFR as a cellular coreceptor. However, it remains possible that AAV3 does not use HSPG as a cellular receptor *in vivo*, even though transduction by AAV3 vectors has been shown to be inhibited by heparin *in vitro* (Blackburn *et al.*, 2006). It should be emphasized that in those experiments, a relatively large dose (100 μg/ml) of heparin was used to achieve partial inhibition. In our studies, on the other hand, use of a low dose of heparin (5 μg/ml) led to an $\sim$2- to 3-fold increase in the transduction efficiency of AAV3 vectors (data not shown).

Although additional studies are warranted to further characterize the precise role of HSPG in the life cycle of AAV3, the evidence presented here that hHGFR is a receptor/coreceptor for AAV3 promises to lead to the development of a useful murine xenograft model to evaluate the safety and efficacy of AAV3 vectors for the potential gene therapy of human hepatoblastoma and hepatocellular carcinoma, especially because AAV3 vectors efficiently transduce primary human hepatocytes, and because transgene expression can be restricted only to liver cancer cells (Glushakova *et al.*, 2009). This possibility has gained further support from our observations that site-directed mutagenesis of surface-exposed tyrosine residues in the AAV3 capsid further improves the transduction efficiency of AAV3 serotype vectors in human liver cancer cells as well (our unpublished data), which is consistent with our published studies with tyrosine-mutant AAV2, AAV6, AAV8, and AAV9 serotype vectors (Zhong *et al.*, 2008;

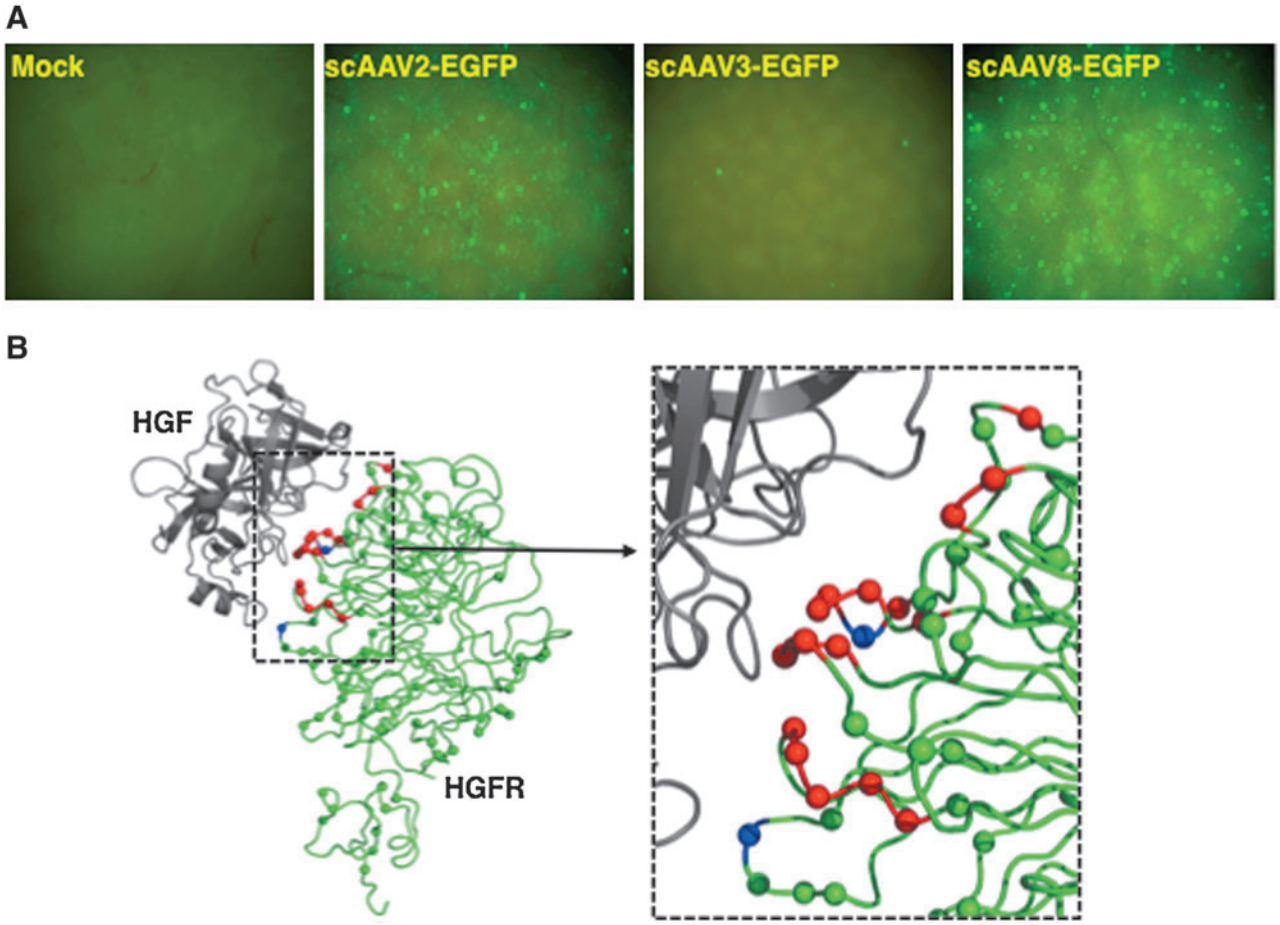

FIG. 3. (**A**) Transduction efficiency of scAAV2, scAAV3, and scAAV8 serotype vectors in murine hepatocytes *in vivo*. Male C57BL/6J mice were either mock-injected, or injected with 1×10^{10} VG each of scAAV2-CBAp-EGFP, scAAV3-CBAp-EGFP, or scAAV8-CBAp-EGFP vectors via the tail vein. Eight weeks postinjection, liver tissues were harvested and sections of each of the lobes were examined for EGFP expression, using a fluorescence microscope. Original magnification,×100. (**B**) Crystal structure of the HGF β chain (gray) in complex with the Sema (extracellular) domain of hHGFR (green). In hHGFR, the C_α positions of amino acids that differ from mHGFR are depicted as green spheres; the amino acids that contact HGF in the crystal structure are depicted as red spheres; and amino acids that differ between hHGFR and mHGFR, and also make contact with HGF, are depicted as blue spheres. A close-up of the HGF and hHGFR contact region is also shown. The RCSB Protein Data Bank accession code for the coordinates used for this image is 1SHY. The program used to generate this image was PyMol.

Petrs-Silva *et al.*, 2009; Jayandharan *et al.*, 2010; Kauss *et al.*, 2010; Li *et al.*, 2010; Markusic *et al.*, 2010; Ojano-Dirain *et al.*, 2010; Petrs-Silva *et al.*, 2010; Qiao *et al.*, 2010).

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Author Disclosure Statement

None of the authors has any competing financial interest, or anything to disclose.

References

Akache, B., Grimm, D., Pandey, K., Yant, S.R., Xu, H., and Kay, M.A. (2006). The 37/67-kilodalton laminin receptor is a receptor for adeno-associated virus serotypes 8, 2, 3, and 9. J. Virol. 80, 9831–9836.

Asokan, A., Hamra, J.B., Govindasamy, L., Agbandje-McKenna, M., and Samulski, R.J. (2006). Adeno-associated virus type 2 contains an integrin $\alpha_5\beta_1$ binding domain essential for viral cell entry. J. Virol. 80, 8961–8969.

Auricchio, A., Hildinger, M., O'Connor, E., Gao, G.P., and Wilson, J.M. (2001). Isolation of highly infectious and pure

adeno-associated virus type 2 vectors with a single-step gravity-flow column. Hum. Gene Ther. 12, 71–76.

Blackburn, S.D., Steadman, R.A., and Johnson, F.B. (2006). Attachment of adeno-associated virus type 3H to fibroblast growth factor receptor 1. Arch. Virol. 151, 617–623.

Chen, T.T., Rakheja, D., Hung, J.Y., Hornsby, P.J., Tabaczewski, P., Malogolowkin, M., Feusner, J., Miskevich, F., Schultz, R., and Tomlinson, G.E. (2009). Establishment and characterization of a cancer cell line derived from an aggressive childhood liver tumor. Pediatr. Blood Cancer 53, 1040–1047.

Chiorini, J.A., Yang, L., Liu, Y., Safer, B., and Kotin, R.M. (1997). Cloning of adeno-associated virus type 4 (AAV4) and generation of recombinant AAV4 particles. J. Virol. 71, 6823–6833.

Chiorini, J.A., Kim, F., Yang, L., and Kotin, R.M. (1999). Cloning and characterization of adeno-associated virus type 5. J. Virol. 73, 1309–1319.

Daya, S., and Berns, K.I. (2008). Gene therapy using adeno-associated virus vectors. Clin. Microbiol. Rev. 21, 583–593.

Di Pasquale, G., Davidson, B.L., Stein, C.S., Martins, I., Scudiero, D., Monks, A., and Chiorini, J.A. (2003). Identification of PDGFR as a receptor for AAV-5 transduction. Nat. Med. 9, 1306–1312.

Gao, G., Vandenberghe, L.H., Alvira, M.R., Lu, Y., Calcedo, R., Zhou, X., and Wilson, J.M. (2004). Clades of adeno-associated viruses are widely disseminated in human tissues. J. Virol. 78, 6381–6388.

Gao, G.P., Alvira, M.R., Wang, L., Calcedo, R., Johnston, J., and Wilson, J.M. (2002). Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. Proc. Natl. Acad. Sci. U.S.A. 99, 11854–11859.

Glushakova, L.G., Lisankie, M.J., Eruslanov, E.B., Ojano-Dirain, C., Zolotukhin, I., Liu, C., Srivastava, A., and Stacpoole, P.W. (2009). AAV3-mediated transfer and expression of the pyruvate dehydrogenase E1α subunit gene causes metabolic remodeling and apoptosis of human liver cancer cells. Mol. Genet. Metab. 98, 289–299.

Grigioni, W.F., Fiorentino, M., D'Errico, A., Ponzetto, A., Crepaldi, T., Prat, M., and Comoglio, P.M. (1995). Overexpression of c-*met* protooncogene product and raised Ki67 index in hepatocellular carcinomas with respect to benign liver conditions. Hepatology 21, 1543–1546.

Jayandharan, G.R., Zhong, L., Sack, B.K., Rivers, A.E., Li, M., Li, B., Herzog, R., and Srivastava, A. (2010). Optimized adeno-associated virus (AAV)-protein phosphatase-S helper viruses for efficient liver transduction by single-stranded AAV vectors: therapeutic expression of factor IX at reduced vector doses. Hum. Gene Ther. 21, 271–283.

Kaludov, N., Brown, K.E., Walters, R.W., Zabner, J., and Chiorini, J.A. (2001). Adeno-associated virus serotype 4 (AAV4) and AAV5 both require sialic acid binding for hemagglutination and efficient transduction but differ in sialic acid linkage specificity. J. Virol. 75, 6884–6893.

Kashiwakura, Y., Tamayose, K., Iwabuchi, K., Hirai, Y., Shimada, T., Matsumoto, K., Nakamura, T., Watanabe, M., Oshimi, K., and Daida, H. (2005). Hepatocyte growth factor receptor is a coreceptor for adeno-associated virus type 2 infection. J. Virol. 79, 609–614.

Kauss, M.A., Smith, L.J., Zhong, L., Srivastava, A., Wong, K.K. Jr., and Chatterjee, S. (2010). Enhanced long-term transduction and multilineage engraftment of human hematopoietic stem cells transduced with tyrosine-modified recombinant adeno-associated virus serotype 2. Hum. Gene Ther. 21, 1129–1136.

Kube, D.M., and Srivastava, A. (1997). Quantitative DNA slot blot analysis: Inhibition of DNA binding to membranes by magnesium ions. Nucleic Acids Res. 25, 3375–3376.

Kurzeder, C., Koppold, B., Sauer, G., Pabst, S., Kreienberg, R., and Deissler, H. (2007). CD9 promotes adeno-associated virus type 2 infection of mammary carcinoma cells with low cell surface expression of heparan sulphate proteoglycans. Int. J. Mol. Med. 19, 325–333.

Li, M., Jayandharan, G.R., Li, B., Ling, C., Ma, W., Srivastava, A., and Zhong, L. (2010). High-efficiency transduction of fibroblasts and mesenchymal stem cells by tyrosine-mutant AAV2 vectors for their potential use in cellular therapy. Hum. Gene Ther. 21, 1527–1543.

Markusic, D.M., Herzog, R.W., Aslanidi, G.V., Hoffman, B.E., Li, B., Li, M., Jayandharan, G.R., Ling, C., Zolotukhin, I., Ma, W., Zolotukhin, S., Srivastava, A., and Zhong, L. (2010). High-efficiency transduction and correction of murine hemophilia B using AAV2 vectors devoid of multiple surface-exposed tyrosines. Mol. Ther. Aug 24. [Epub ahead of print] PMID: 20736929.

Muramatsu, S., Mizukami, H., Young, N.S., and Brown, K.E. (1996). Nucleotide sequencing and generation of an infectious clone of adeno-associated virus 3. Virology 221, 208–217.

Nakabayashi, H., Taketa, K., Miyano, K., Yamane, T., and Sato, J. (1982). Growth of human hepatoma cells lines with differentiated functions in chemically defined medium. Cancer Res. 42, 3858–3863.

Ojano-Dirain, C., Glushakova, L.G., Zhong, L., Zolotukhin, S., Muzyczka, N., Srivastava, A., and Stacpoole, P.W. (2010). An animal model of PDH deficiency using AAV8-siRNA vector-mediated knockdown of pyruvate dehydrogenase E1α. Mol. Genet. Metab. 101, 183–191.

Okano, J., Shiota, G., and Kawasaki, H. (1999). Expression of hepatocyte growth factor (HGF) and HGF receptor (c-Met) proteins in liver diseases: An immunohistochemical study. Liver 19, 151–159.

Petrs-Silva, H., Dinculescu, A., Li, Q., Min, S.H., Chiodo, V., Pang, J.J., Zhong, L., Zolotukhin, S., Srivastava, A., Lewin, A.S., and Hauswirth, W.W. (2009). High-efficiency transduction of the mouse retina by tyrosine-mutant AAV serotype vectors. Mol. Ther. 17, 463–471.

Petrs-Silva, H., Dinculescu, A., Li, Q., Deng, W.T., Pang, J.J., Min, S.H., Chiodo, V., Neeley, A.W., Govindasamy, L., Bennett, A., Agbandje-McKenna, M., Zhong, L., Li, B., Jayandharan, G.R., Srivastava, A., Lewin, A.S., and Hauswirth, W.W. (2010). Novel properties of tyrosine-mutant AAV2 vectors in the mouse retina. Mol. Ther. Nov 2. [Epub ahead of print] PMID: 21045809.

Ponnazhagan, S., Wang, X.S., Woody, M.J., Luo, F., Kang, L.Y., Nallari, M.L., Munshi, N.C., Zhou, S.Z., and Srivastava, A. (1996). Differential expression in human cells from the p6 promoter of human parvovirus B19 following plasmid transfection and recombinant adeno-associated virus 2 (AAV) infection: Human megakaryocytic leukaemia cells are non-permissive for AAV infection. J. Gen. Virol. 77, 1111–1122.

Qiao, C., Zhang, W., Yuan, Z., Shin, J.H., Li, J., Jayandharan, G.R., Zhong, L., Srivastava, A., Xiao, X., and Duan, D. (2010). Adeno-associated virus serotype 6 capsid tyrosine-to-phenylalanine mutations improve gene transfer to skeletal muscle. Hum. Gene Ther. 21, 1343–1348.

Qing, K., Mah, C., Hansen, J., Zhou, S., Dwarki, V., and Srivastava, A. (1999). Human fibroblast growth factor receptor 1 is a co-receptor for infection by adeno-associated virus 2. Nat. Med. 5, 71–77.

Rabinowitz, J.E., Bowles, D.E., Faust, S.M., Ledford, J.G., Cunningham, S.E., and Samulski, R.J. (2004). Cross-dressing the virion: The transcapsidation of adeno-associated virus serotypes functionally defines subgroups. J. Virol. 78, 4421–4432.

Rong, S., Bodescot, M., Blair, D., Dunn, J., Nakamura, T., Mizuno, K., Park, M., Chan, A., Aaronson, S., and Vande Woude, G.F. (1992). Tumorigenicity of the *met* proto-oncogene and the gene for hepatocyte growth factor. Mol. Cell. Biol. 12, 5152–5158.

Rutledge, E.A., Halbert, C.L., and Russell, D.W. (1998). Infectious clones and vectors derived from adeno-associated virus (AAV) serotypes other than AAV type 2. J. Virol. 72, 309–319.

Stamos, J., Lazarus, R.A., Yao, X., Kirchhofer, D., and Wiesmann, C. (2004). Crystal structure of the HGF β-chain in complex with the Sema domain of the Met receptor. EMBO J. 23, 2325–2335.

Summerford, C., and Samulski, R.J. (1998). Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. J. Virol. 72, 1438–1445.

Summerford, C., Bartlett, J.S., and Samulski, R.J. (1999). $\alpha_V\beta_5$ integrin: A co-receptor for adeno-associated virus type 2 infection. Nat. Med. 5, 78–82.

Thomas, C.E., Storm, T.A., Huang, Z., and Kay, M.A. (2004). Rapid uncoating of vector genomes is the key to efficient liver transduction with pseudotyped adeno-associated virus vectors. J. Virol. 78, 3110–3122.

Walters, R.W., Yi, S.M., Keshavjee, S., Brown, K.E., Welsh, M.J., Chiorini, J.A., and Zabner, J. (2001). Binding of adeno-associated virus type 5 to 2,3-linked sialic acid is required for gene transfer. J. Biol. Chem. 276, 20610–20616.

Weller, M.L., Amornphimoltham, P., Schmidt, M., Wilson, P.A., Gutkind, J.S., and Chiorini, A. (2010). Epidermal growth factor receptor is a co-receptor for adeno-associated virus serotype 6. Nat. Med. 16, 662–664.

Wu, J., Zhao, W., Zhong, L., Han, Z., Li, B., Ma, W., Weigel-Kelley, K.A., Warrington, K.H., and Srivastava, A. (2007). Self-complementary recombinant adeno-associated viral vectors: Packaging capacity and the role of Rep proteins in vector purity. Hum. Gene Ther. 18, 171–182.

Wu, Z., Miller, E., Agbandje-McKenna, M., and Samulski, R.J. (2006). α2,3 and α2,6 N-linked sialic acids facilitate efficient binding and transduction by adeno-associated virus types 1 and 6. J. Virol. 80, 9093–9103.

Zhong, L., Li, B., Mah, C.S., Govindasamy, L., Agbandje-McKenna, M., Cooper, M., Herzog, R.W., Zolotukhin, I., Warrington, K.H., Jr., Weigel-Van Aken, K.A., Hobbs, J.A., Zolotukhin, S., Muzyczka, N., and Srivastava, A. (2008). Next generation of adeno-associated virus 2 vectors: Point mutations in tyrosines lead to high-efficiency transduction at lower doses. Proc. Natl. Acad. Sci. U.S.A. 105, 7827–7832.

Zincarelli, C., Soltys, S., Rengo, G., and Rabinowitz, J.E. (2008). Analysis of AAV serotypes 1–9 mediated gene expression and tropism in mice after systemic injection. Mol. Ther. 16, 1073–1080.

Address correspondence to:
Dr. Arun Srivastava
Division of Cellular and Molecular Therapy
Cancer and Genetics Research Complex
2033 Mowry Road, Room 492-A
Gainesville, FL 32610-3633

E-mail: aruns@peds.ufl.edu

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