

## Ex Vivo Serotype-Specific Transduction of Equine Joint Tissue by Self-Complementary Adeno-Associated Viral Vectors

L.R. Goodrich,<sup>1</sup> V.W. Choi,<sup>2</sup> B.A. Duda Carbone,<sup>1</sup> C.W. McIlwraith,<sup>1</sup> and R.J. Samulski<sup>3</sup>

### Abstract

Cell transplantation for the treatment of joint disease is an important clinical tool. Genetic modification of cells before transplantation has shown enhanced healing. *Ex vivo* genetic modification of joint tissue cells with various adeno-associated virus (AAV) serotypes has not been investigated. The transduction efficiencies of self-complementary AAV serotypes (1–6 and 8) were determined in joint tissue containing chondrocytes and synoviocytes isolated from equine models. When comparing scAAV serotypes for efficient transduction *ex vivo*, in chondrocytes versus synoviocytes, serotypes 6 and 2, and serotypes 3 and 2, respectively, appeared superior for gene expression. Unlike adenoviral vectors, no upregulation of inflammatory markers, such as matrix metalloproteinases and aggrecanase, was seen on treatment of joint tissue with AAV vectors *ex vivo*. Our findings also corroborate that *ex vivo* transduction of joint tissue can result in high transgene protein levels over time, and transplantation modalities might be feasible using AAV vectors in the treatment of joint-related diseases.

THE FIELD OF GENE THERAPY has shown much promise in the area of musculoskeletal diseases, specifically arthritis (Evans and Robbins, 1994; Ghivizzani *et al.*, 1998; Goater *et al.*, 2000; Kafienah *et al.*, 2003; Evans, 2004; Evans *et al.*, 2004; Goodrich *et al.*, 2006, 2007). There are myriad proteins that have been identified as decreasing catabolism and increasing anabolism in joints through targeting the main cell types, chondrocytes and synoviocytes (Ulrich-Vinther, 2007). Gene therapy using both *in vivo* and *ex vivo* approaches offers the potential of longer term protein expression than recombinant proteins and benefits such as steady state protein expression, delivery of proteins to cells surrounded by matrix such as chondrocytes in the deep layers of cartilage, and higher overall levels of therapeutic proteins. In addition, further development of stem cell therapy strengthens the need to maximize *ex vivo* gene delivery.

Various viral and nonviral vectors have been studied in the field of gene therapy for arthritis (Evans, 2004; Evans *et al.*, 2004; Gouze *et al.*, 2002, 2003, 2004). Recombinant adeno-associated viral (AAV) vectors are highly attractive vectors for the treatment of joint diseases by virtue of their ability to promote long-term expression and to infect non-dividing cells, and because of the broad tissue tropism exhibited by AAV serotypes (Ulrich-Vinther, 2007). To date, most studies of joint tissues have used recombinant AAV (rAAV) serotype 2 (Madry *et al.*, 2003, 2005; Cucchiariini and Madry, 2005; Ulrich-Vinther *et al.*, 2005; Dai and Rabie,

2007a). However, the prevalence of antibodies against this serotype is high in the human population (Grimm and Kay, 2003) and one study by Adriaansen and colleagues reported a significantly greater immune response to rAAV2 compared with rAAV5 when injected into the ankle joints of rats (Adriaansen *et al.*, 2005). At present, at least 12 naturally occurring serotypes of AAV have been isolated, cloned, and sequenced (AAV1 to AAV12) (Choi *et al.*, 2005; Gao *et al.*, 2005; Schmidt *et al.*, 2008). Studies conducted in various skeletal tissues have revealed a wide array of tissue transduction efficiencies depending on serotype (Riviere *et al.*, 2006; Dai and Rabie, 2007b). In 2001, McCarty and colleagues reported increased efficacy of rAAV vectors by synthesizing a self-complementary recombinant AAV vector (scAAV) that spontaneously reanneals, alleviating the requirement rAAV has for host cell DNA synthesis (McCarty *et al.*, 2001). Bypassing rate-limiting second-strand DNA synthesis within the host cell resulted in 5- to 140-fold more efficient transduction than conventional rAAV (McCarty *et al.*, 2001). This step becomes more important when considering cell types that have little to no DNA replication taking place (i.e., neurons, cardiomyocytes, and chondrocytes). This has direct implications for the successful use of scAAV vectors to treat joint diseases because the cells of joints have a low rate of cell division.

Previous studies have shown that *ex vivo* genetic modification of cells before implantation can enhance cartilage

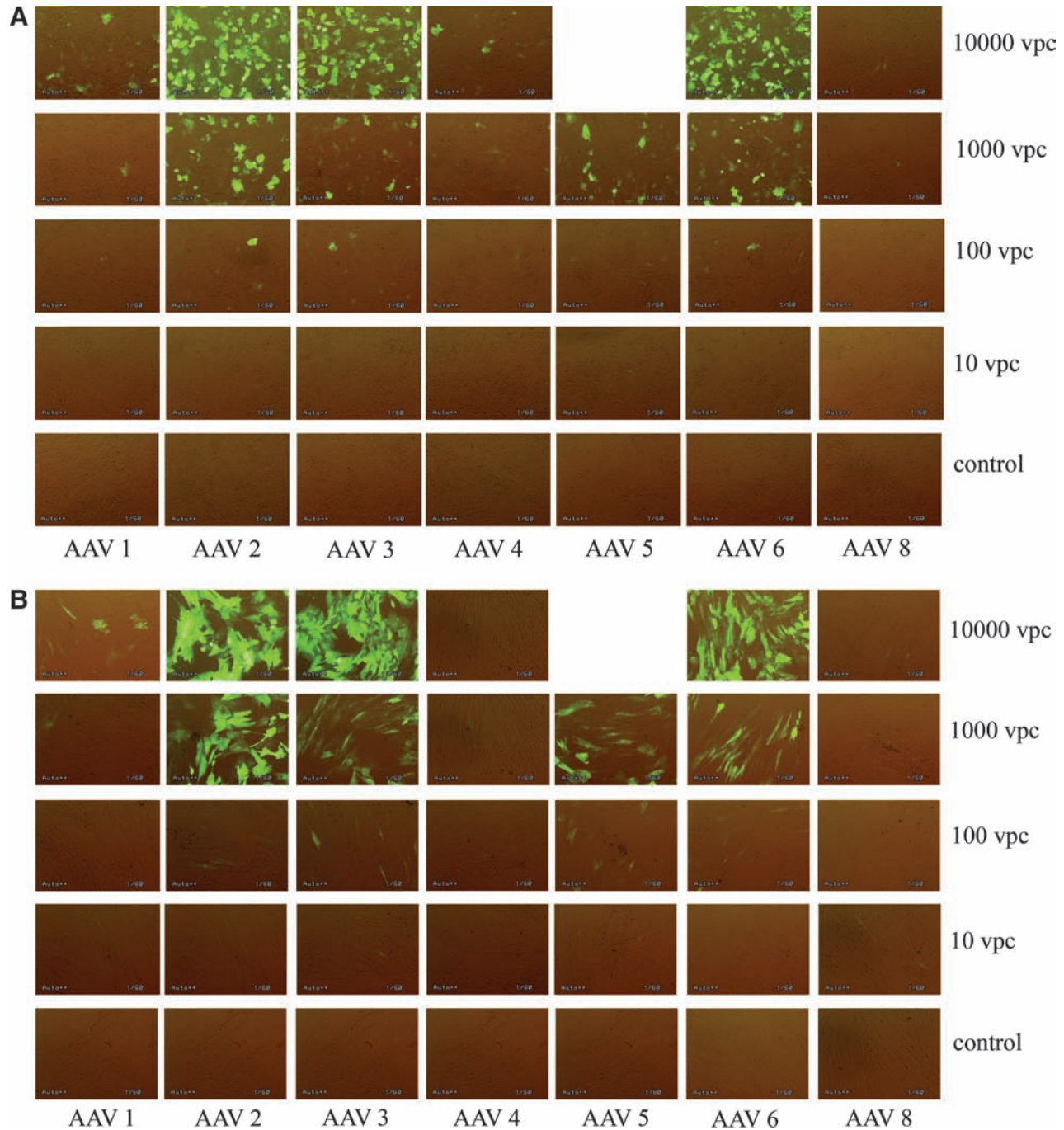
<sup>1</sup>Orthopaedic Research Center, College of Veterinary Medicine, Colorado State University, Fort Collins, CO 80523.

<sup>2</sup>Novartis Institute for Biomedical Research, Cambridge, MA 02139.

<sup>3</sup>University of North Carolina Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

healing (Hidaka *et al.*, 2003; Goodrich *et al.*, 2005, 2007; Madry *et al.*, 2005). No investigations have been performed that evaluate the efficacy of various scAAV serotypes *ex vivo*, before transplantation. Typically, these cells are implanted into a cartilage defect 48–72 hr after transduction (Hidaka *et al.*, 2003; Goodrich *et al.*, 2007). Establishment of the appropriate scAAV serotype to maximize transduction of these cells would be of great interest to clinicians and researchers using this important clinical tool.

In an effort to determine the maximal *ex vivo* transduction efficiency of scAAV vectors in joint tissues, we tested scAAV serotypes 1, 2, 3, 4, 5, 6, and 8 (scAAV1–6 and scAAV8) in chondrocytes and synoviocytes. Initial screening tests were first performed to evaluate transduction. Further testing was performed with optimal serotypes to evaluate transduction efficiency, appropriate viral dose, potential cell toxicity, length of protein expression, and cytokine response to serotypes. To the best of our knowledge, this is the first study to



**FIG. 1.** Initial screening of scAAVGFP1, 2, 3, 4, 5, 6 and 8 in (A) chondrocytes and (B) synoviocytes in monolayer cell culture on day 7. scAAV2, 3, 5, and 6 had the greatest amount of GFP expression. The viral dose of 10,000 viral particles per cell (VPC) had the highest percentage transduction. Bottom labels indicate serotypes and right column labels indicate the VPC. Original magnification,  $\times 40$ .

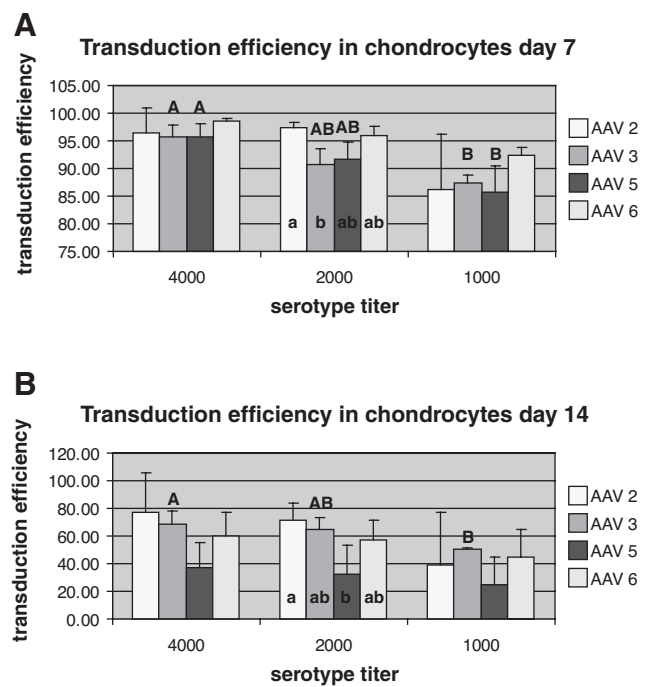
evaluate scAAV serotypes in chondrocytes and synoviocytes. The data described herein will be relevant for the design of future *ex vivo* transduction studies involving scAAV vectors in therapeutic gene transfer to joint tissue.

All scAAV vectors used in this study were provided by the Gene Therapy Center Vector Core Facility (Chapel Hill, CA). The scAAVGFP construct (pHpa-trs-SK) used in this study has been described previously (McCarty *et al.*, 2001) and cross-packaging in various serotype capsids was performed as described (Rabinowitz *et al.*, 2002). Statistical analysis involved one-way analysis of variance (ANOVA) of all continuous data with Tukey post-hoc comparison when statistical significance was detected. Ordinal data (viability and cells scores) were analyzed by Kruskal–Wallis test. Statistical significance was determined when  $p \leq 0.05$  and a trend was noted when  $0.05 < p < 0.1$ .

For preliminary screening of AAV serotypes, chondrocytes and synoviocytes were harvested from femoropatellar joints of neonatal foals (1 to 2 days old) at postmortem, and stored in liquid nitrogen until ready for use (Nixon *et al.*, 1992). Cells were thawed for plating as previously described (Brower-Toland *et al.*, 2001; Saxer *et al.*, 2001) and monolayer cultures in HEPES-buffered F-12 medium with 10% fetal bovine serum (FBS) (GIBCO, Grand Island, NY) at 50% confluence were established in 24-well plates. One day after seeding, cells were transduced with scAAV1–6 and scAAV8 packaging the green fluorescent protein (GFP)-encoding transgene driven by the cytomegalovirus (CMV) promoter at various levels of viral particles per cell (VPC). Fluorescence micrographs were obtained every 3 days for the first 30 days and then weekly for up to 90 days to monitor GFP expression. As shown in Fig. 1, preliminary screening of scAAVGFP serotypes revealed that scAAV2, 3, 5, and 6 transduced chondrocyte/synoviocyte cultures at high efficiency by day 7. scAAV1, 4, and 8 had minimal GFP expression at all of the viral doses tested in both chondrocytes and synoviocytes. Transduction efficiencies of serotypes at 10,000 VPC followed the order scAAV2 > scAAV6 > scAAV3 in chondrocytes (Fig. 1A) and scAAV2 > scAAV3 > scAAV6 in synoviocytes (Fig. 1B). Although scAAV5GFP was not tested at this VPC dose (due to low titer), these vectors appeared to transduce joint tissue with efficiency similar to scAAV3 and scAAV6 vectors at 1000 VPC.

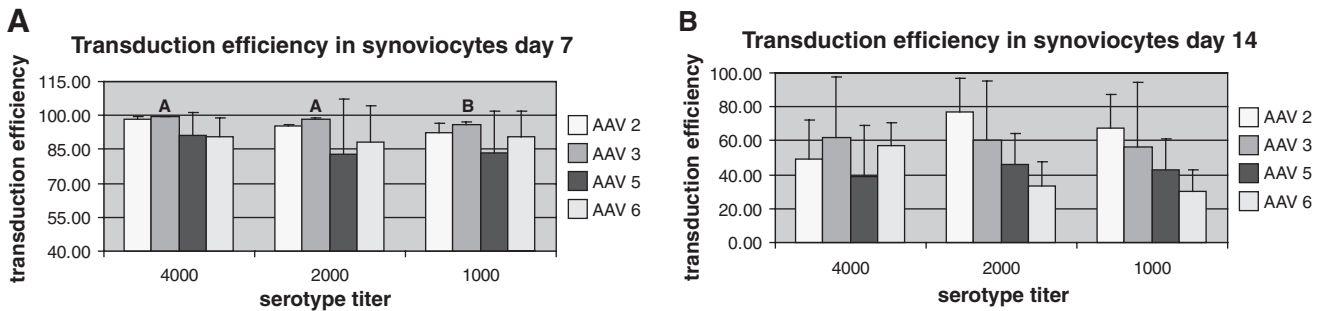
This observed trend is in general agreement with other studies that demonstrate serotype selectivity in skeletal muscle (Riviere *et al.*, 2006) or chondrocytes and bone marrow-derived mesenchymal stem cells (Dai and Rabie, 2007a). In a later study by Dai and colleagues, however, rAAV serotype 1 appeared to transduce rat chondrocytes efficiently (Dai and Rabie, 2007b), which is in contrast to our findings in the equine model. This can likely be attributed to species-specific differences in tissue tropism of AAV serotypes (Duan *et al.*, 2000; Sanlioglu *et al.*, 2001), and stresses the need to test various AAV serotypes in the species of interest before testing therapeutic transgenes.

On the basis of this preliminary screening, we quantitated the transduction efficiency of scAAV2, 3, 5, and 6 in chondrocytes and synoviocytes by flow cytometry-assisted cell sorting on days 7 and 14 posttransduction. Briefly, sorting was conducted with a MoFlo cell sorter/analyzer (Beckman Coulter, Fullerton, CA) with a 100- $\mu$ m flow cell tip used at a sheath pressure of 30 psi and a flow rate of 12,000 events per



**FIG. 2.** Quantitative analysis (flow cytometry-assisted cell sorting) of the transduction efficiency of scAAVGFP2, 3, 5, and 6. Transduction was performed on chondrocytes in monolayer at (A) 7 days and (B) 14 days. Flow cytometry was performed on days 7 and 14 after transduction with 4000, 2000, and 1000 VPC. Capital letters denote significant differences in titer within serotypes, and uncapitalized letters denote significant differences between serotypes within each titer tested.

second, with a laser line of 488 nm and a laser power of 110. Cells were sorted on the basis of GFP fluorescence with a 530/540 bandpass filter, preceded by a neutral-density 2.0 filter and high voltage of 400–450 with a log signal. Summit software (version 4.0; Beckman Coulter) was used to collect histograms and set sort parameters. As shown in Fig. 2A, on day 7, 95–99% GFP-positive cells were detected for scAAV2, 3, 5, and 6 at 4000 VPC in chondrocytes. No significant differences were seen in transduction efficiencies between the serotypes (~85–90% GFP-positive cells) for scAAV2, 3, 5, and 6 at a dose of 4000 VPC. Of the dosing groups, AAV3 and AAV5 had significantly better transduction efficiencies at 4000 VPC than at 1000 VPC and within the dosing groups, AAV2 at 2000 VPC had significantly greater transducing efficiency than AAV3. However, as seen in Fig. 2B, analysis of samples on day 14 revealed a statistically significant lower transduction efficiency for scAAV5 (~36% GFP-positive cells) in comparison with scAAV2 at 2000 VPC. Dose did not affect serotype transduction efficiency on day 14 except for AAV3, for which 4000 VPC was better than 1000 VPC. In synoviocytes, a transduction efficiency of 85–100% (GFP-positive cells) was seen for each serotype tested on day 7 posttransduction (Fig. 3A). AAV3 had a higher transduction efficiency at 4000 and 2000 VPC than at 1000 VPC. At 14 days there were no significant differences between AAV serotypes 2, 3, 5, and 6 at any dose. Further, there were no significant differences within any serotype at doses of 4000, 2000, and 1000 VPC (Fig. 3B). For scAAV2, 3, 5, and 6, fluorescence appeared to begin



**FIG. 3.** Quantitative analysis (flow cytometry-assisted cell sorting) of the transduction efficiency of scAAVGFP2, 3, 5, and 6. Transduction was performed on synoviocytes in monolayer at (A) 7 days and (B) 14 days. Flow cytometry was performed on days 7 and 14 after transduction with 4000, 2000, and 1000 VPC. Capital letters denote significant differences in titers within serotypes.

approximately 2 days after transduction and reached maximal fluorescence at approximately 5 days after transduction.

At 14 days both chondrocytes and synoviocytes were approximately doubled in number from day 7, which accounts for the 50% decrease in the number of cells emitting GFP. This decrease in GFP-positive cell number over time can be attributed to dilution of AAV vector genomes and is expected because of the persistence of AAV vector genomes in episomal rather than integrated form (Bartlett *et al.*, 2000). At 51 days, both chondrocytes and synoviocytes had at least 30% GFP-positive cells when transduced with AAV2 or AAV6 (data not shown). This long-term expression is encouraging for therapeutic gene transfer applications in cartilage healing, osteoarthritis, and rheumatoid arthritis, for which the healing time required is prolonged compared with soft tissue (months vs. days) (Sams and Nixon, 1993; Hendrickson *et al.*, 1994).

For cell viability studies, trypan blue staining and morphological studies were carried out on days 0, 3, 7, 10, and 14 posttransduction. Cells were given a score of 0 through 5 on the basis of morphological characteristics. Abnormal morphological characteristics were considered to be rounding, crenation, or lifting off the plate. A score of 1 = 1–20% of the cells had abnormal cell morphology, 2 = 21–40, 3 = 41–60, 4 = 61–80, and 5 = 81–100%. Five views at  $\times 200$  magnification were assessed and an average score was determined. As outlined in Table 1, scAAV2 vectors appear to have some negative impact on both chondrocytes and synoviocytes, based on the observation that several cells became rounded, crenated, or detached from the plate. A trend ( $0.05 < p < 0.1$ ) was detected on days 7, 10, and 14, wherein scAAV2GFP had the highest score (greatest morbidity) at each time point. The reason behind this effect is not clear. No significant differences were detected for viability between any serotype or titer.

**TABLE 1.** AVERAGE CELL VIABILITY AND CELL SCORES<sup>a</sup> FOR CHONDROCYTES AND SYNOVIOCYTES OVER 14 DAYS

Day	scAAV2				scAAV3				scAAV5			
	Control	4000 VPC	2000 VPC	1000 VPC	Control	4000 VPC	2000 VPC	1000 VPC	Control	4000 VPC	2000 VPC	1000 VPC
<b>Chondrocyte Morphology Scores</b>												
3	0 (0)	1.7 (2.1)	0.7 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.7 (1.2)	0 (0)	0 (0)
7	0 (0)	2.7 (1.2) <sup>b</sup>	2.3 (0.6) <sup>b</sup>	2.0 (1)	0 (0)	0.3 (0.6)	0.3 (0.6)	0.7 (0.6)	0 (0)	0.3 (0.6)	0 (0)	0.3 (0.6)
10	0 (0)	3 (1) <sup>b</sup>	2.7 (1.2) <sup>b</sup>	2.3 (1.5)	0 (0)	1.3 (0.6)	1.0 (0)	1.0 (0)	0 (0)	1.3 (1.2)	1 (1.0)	1 (1.0)
14	0 (0)	3 (1) <sup>b</sup>	2.7 (1.2) <sup>b</sup>	2.3 (1.5)	0 (0)	1.3 (0.6)	0.7 (0.6)	0.7 (0.6)	0 (0)	1.0 (1.0)	0.7 (0.6)	0.7 (0.6)
<b>Chondrocyte Viability</b>												
7	94 (2)	81 (21)	87 (5)	94 (2)	90 (7)	94 (3)	94 (4)	91 (5)	96 (4)	85 (9)	86 (14)	73 (17)
14	91 (9)	79 (20)	85 (5)	94 (5)	86 (10)	91 (5)	83 (14)	88 (3)	85 (8)	93 (6)	92 (7)	79 (16)
<b>Synoviocyte Morphology Scores</b>												
3	0 (0)	2.7 (0.6) <sup>c</sup>	1.7 (0.6) <sup>b</sup>	1.0 (1.0)	0 (0)	1.0 (1.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
7	0 (0)	2.7 (0.6) <sup>c</sup>	2.0 (0) <sup>b</sup>	1.3 (0.6)	0 (0)	0.3 (0.6)	0 (0)	0.3 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)
10	0 (0)	2.3 (1.2) <sup>c</sup>	1.7 (0.6) <sup>b</sup>	1.3 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.6)	0.3 (0.6)	0 (0)
14	0 (0)	2.0 (1.0) <sup>c</sup>	1.7 (0.6) <sup>b</sup>	1.3 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.6)	0.3 (0.6)	0 (0)
<b>Synoviocyte Viability</b>												
7	92 (2)	66 (18)	81 (9)	80 (22)	94 (2)	83 (12)	87 (6)	85 (3)	90 (6)	81 (13)	87 (5)	95 (4)
14	85 (4)	63 (28)	83 (14)	75 (17)	85 (11)	83 (15)	79 (11)	73 (18)	77 (10)	67 (14)	68 (30)	36 (12)

Abbreviations: scAAV2, 3, and 5; self-complementary AAV serotypes 2, 3, and 5; VPC = viral particles per cell.

<sup>a</sup>Results are given with standard deviation in parentheses.

<sup>b</sup>A trend ( $0.05 < p < 0.1$ ) toward significant morphological changes compared with control cells.

<sup>c</sup>A significant ( $p < 0.05$ ) morphological change compared with control cells.

Last, matrix metalloproteinases (MMPs) and aggrecanase levels are considered a good representation of inflammatory changes in joint tissues (Ishiguro and Kojima, 2004). An increase in these molecules has been noted with adenoviral vectors (Goodrich *et al.*, 2007). Therefore, in an effort to detect any inflammatory response to AAV serotypes, we carried out quantitative RT-PCR assays to monitor the impact of AAV vectors on expression levels of equine MMP-1, MMP-3, MMP-13, and aggrecanase-1, markers of the inflammatory response in joint tissue. Briefly, RNA was extracted from cells on day 7, using a QIAshredder and RNeasy mini kit (Qiagen, Valencia, CA), and complementary DNA was generated using 50 ng of total RNA and oligo(dT) primers with a SuperScript III first-strand synthesis kit (Invitrogen, Carlsbad, CA). mRNA expression of target genes was compared with the expression levels of equine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as control. Equine MMP-1, MMP-3, MMP-13, and GAPDH primer/probes were obtained from the Lucy Whittier Molecular & Diagnostic Core Facility (University of California Davis, Davis, CA). Primer sequences were as follows: MMP-1 forward, 5'-AAG CTGCTTATGAGGTTTCCCA; reverse, 5'-GGGTATCCGTA GAGCACATCCT; probe, 5'-FAM-AGCCCAGTACTTATTA CCTTTGAAAAACCGGAC-TAMRA-3'; MMP-3 forward, 5'-AACACTGGACGAAGGATGCAT; reverse, 5'-ACCCAGGG AATGACCAAGTTC; probe, 5'-FAM-AGGGATCAATTTTCT CCTTGTGCTGCTCA-TAMRA-3'. Aggrecanase sequences were as follows: forward, 5'-GCCTTCACTGCTGCTCATGA; reverse, 5'-CCAACACATGGCTTTGAATTGT; and probe, 5'-FAM-CTGGGCCATGTCTTCAACATGCTCC-TAMRA-3'. Quantitative RT-PCR was performed, with 2.5 ng of RNA per sample (in duplicate) and a 384-well LightCycler 480 (Roche, Indianapolis, IN). Sample cycle threshold data were normalized to standards for each gene and compared with each other by determining the ratio of the gene of interest to GAPDH. No significant trends were noted in the inflammatory response to AAV vectors, with the overall effects being negligible. Although not statistically significant, a slight increase in aggrecanase RNA expression was noted in both chondrocytes and synoviocytes for scAAV3 vectors. These results seem to be consistent with published observations that AAV, when compared with other DNA viruses (e.g., adenovirus), is dramatically different with respect to the acute immune response (Wu *et al.*, 2006).

In conclusion, this study identified four serotypes of scAAV that are effective in transducing primary chondrocytes and synoviocytes *ex vivo*. This is of significant importance because implantation of primary cells (chondrocytes and synoviocytes) for joint therapy in both human and equine patients is routine for osteochondritis dissecans (OCD) and acute cartilage traumatic lesions and would dramatically benefit from therapeutic gene transfer augmentation (IGF-I, IRAP, and BMP-7) (Marlovits *et al.*, 2004; Fortier and Nixon, 2005). In chondrocytes, scAAV6 and scAAV2 appear to be the most efficient whereas in synoviocytes, scAAV3 and scAAV2 were more robust. With the identification of surface receptors for AAV1, 2, 4, 5, and 6 (Wu *et al.*, 2006), and the high degree of sequence homology between AAV2 and AAV3 (88%), the potential for this information to facilitate the generation and testing of chimeric AAV particles with improved chondrocyte and synovial cell transduction is imminent (Wu *et al.*, 2006). We have suc-

cessfully transplanted chondrocytes in a lesion in equine joints and tracked cell viability out to 9 weeks (Goodrich *et al.*, 2005). These observations and our findings that self-complementary AAV can efficiently transduce joint tissue *ex vivo*, resulting in high transgene protein levels over time, have set the stage for evaluating genetically modified therapeutic cells for transplantation in diseased joints.

### Acknowledgments

This study was funded by NIH 1K08AR054903-01A2 and by a Colorado State University College Research Council grant.

### Author Disclosure Statement

The authors (L.R. Goodrich, V.W. Choi, B.A. Duda Carbone, C.W. McIlwraith, and R.J. Samulski) do not have any conflicts of interest in the publication of this manuscript.

### References

- Adriaansen, J., Tas, S.W., Klarenbeek, P.L., Bakker, A.C., Apparijly, F., Firestein, G.S., Jorgensen, C., Vervoordeldonk, M.J., and Tak, P.P. (2005). Enhanced gene transfer to arthritic joints using adeno-associated virus type: Implications for intra-articular gene therapy. *Ann. Rheum. Dis.* 64, 1677–1684.
- Bartlett, J.S., Wilcher, R., and Samulski, R.J. (2000). Infectious entry pathway of adeno-associated virus and adeno-associated virus vectors. *J. Virol.* 74, 2777–2785.
- Brower-Toland, B.D., Saxer, R.A., Goodrich, L.R., Mi, Z., Robbins, P.D., Evans, C.H., and Nixon, A.J. (2001). Direct adenovirus-mediated insulin-like growth factor I gene transfer enhances transplant chondrocyte function. *Hum. Gene Ther.* 12, 117–129.
- Choi, V.W., McCarty, D.M., and Samulski, R.J. (2005). AAV hybrid serotypes: Improved vectors for gene delivery. *Curr. Gene Ther.* 5, 299–310.
- Cucchiari, M., and Madry, H. (2005). Gene therapy for cartilage defects. *J. Gene Med.* 7, 1495–1509.
- Dai, J., and Rabie, A.B. (2007a). Direct AAV-mediated gene delivery to the temporomandibular joint. *Front. Biosci.* 12, 2212–2220.
- Dai, J., and Rabie, A.B. (2007b). Recombinant adeno-associated virus vector hybrids efficiently target different skeletal cells. *Front. Biosci.* 12, 4280–4287.
- Duan, D., Yue, Y., Yan, Z., Yang, J., and Engelhardt, J.F. (2000). Endosomal processing limits gene transfer to polarized airway epithelia by adeno-associated virus. *J. Clin. Invest.* 105, 1573–1587.
- Evans, C.H. (2004). Gene therapies for osteoarthritis. *Curr. Rheumatol. Rep.* 6, 31–40.
- Evans, C.H., and Robbins, P.D. (1994). Gene therapy for arthritis. In *Gene Therapeutics: Methods and Applications of Direct Gene Transfer*. J.A. Wolff, ed. (Birkhauser, Cambridge) pp. 320–343.
- Evans, C.H., Gouze, J.N., Gouze, E., Robbins, P.D., and Ghivizzani, S.C. (2004). Osteoarthritis gene therapy. *Gene Ther.* 11, 379–389.
- Fortier, L.A., and Nixon, A.J. (2005). New surgical treatments for osteochondritis dissecans and subchondral bone cysts. *Vet. Clin. North Am. Equine Pract.* 21, 673–690, vii.
- Gao, G., Vandenberghe, L.H., and Wilson, J.M. (2005). New recombinant serotypes of AAV vectors. *Curr. Gene Ther.* 5, 285–297.

- Ghivizzani, S.C., Lechman, E.R., Kang, R., Tio, C., Kolls, J., Evans, C.H., and Robbins, P.D. (1998). Direct adenovirus-mediated gene transfer of interleukin 1 and tumor necrosis factor  $\alpha$  soluble receptors to rabbit knees with experimental arthritis has local and distal anti-arthritic effects. *Proc. Natl. Acad. Sci. U.S.A.* 95, 4613–4618.
- Goater, J., Muller, R., Kollias, G., Firestein, G., Sanz, I., O'Keefe, R.J., and Schwarz, E.M. (2000). Empirical advantages of adeno-associated viral vectors in *in vivo* gene therapy for arthritis. *J. Rheumatol.* 27, 983–989.
- Goodrich, L.R., Nixon, A.J., Hidaka, C., Robbins, P.D., and Evans, C.H. (2005). Enhanced early repair and cell survival in cartilage defects implanted with chondrocytes over expressing insulin-like growth factor-I. *Vet. Surg.* 34, E8.
- Goodrich, L.R., Brower-Toland, B.D., Warnick, L., Robbins, P.D., Evans, C.H., and Nixon, A.J. (2006). Direct adenovirus-mediated IGF-I gene transduction of synovium induces persisting synovial fluid IGF-I ligand elevations. *Gene Ther.* 13, 1253–1262.
- Goodrich, L.R., Hidaka, C., Robbins, P.D., Evans, C.H., and Nixon, A.J. (2007). Genetic modification of chondrocytes with insulin-like growth factor-1 enhances cartilage healing in an equine model. *J. Bone Joint Surg. Br.* 89, 672–685.
- Gouze, E., Pawliuk, R., Pilapil, C., Gouze, J.N., Fleet, C., Palmer, G.D., Evans, C.H., Leboulch, P., and Ghivizzani, S.C. (2002). *In vivo* gene delivery to synovium by lentiviral vectors. *Mol. Ther.* 5, 397–404.
- Gouze, E., Pawliuk, R., Gouze, J.N., Pilapil, C., Fleet, C., Palmer, G.D., Evans, C.H., Leboulch, P., and Ghivizzani, S.C. (2003). Lentiviral-mediated gene delivery to synovium: Potent intra-articular expression with amplification by inflammation. *Mol. Ther.* 7, 460–466.
- Gouze, J.N., Gouze, E., Palmer, G.D., Kaneto, H., Ghivizzani, S.C., Grodzinsky, A.J., and Evans, C.H. (2004). Adenovirus-mediated gene transfer of glutamine: Fructose-6-phosphate amidotransferase antagonizes the effects of interleukin-1 $\beta$  on rat chondrocytes. *Osteoarthritis Cartilage* 12, 217–224.
- Grimm, D., and Kay, M.A. (2003). From virus evolution to vector revolution: Use of naturally occurring serotypes of adeno-associated virus (AAV) as novel vectors for human gene therapy. *Curr. Gene Ther.* 3, 281–304.
- Hendrickson, D.A., Nixon, A.J., Grande, D.A., Todhunter, R.J., Minor, R.R., Erb, H., and Lust, G. (1994). Chondrocyte-fibrin matrix transplants for resurfacing extensive articular cartilage defects. *J. Orthop. Res.* 12, 485–497.
- Hidaka, C., Goodrich L.R., Chen, C.T., Warren R.F., Crystal, R.G., and Nixon, A.J. (2003). Acceleration of cartilage repair by genetically modified chondrocytes over expressing bone morphogenetic protein-7. *J. Orthop. Res.* 21, 573–583.
- Ishiguro, N., and Kojima, T. (2004). [Role of aggrecanase and MMP in cartilage degradation]. *Clin. Calcium* 14, 38–44.
- Kafienah, W., Al Fayed, F., Hollander, A.P., and Barker, M.D. (2003). Inhibition of cartilage degradation: A combined tissue engineering and gene therapy approach. *Arthritis Rheum.* 48, 709–718.
- Madry, H., Cucchiari, M., Terwilliger, E.F., and Trippel, S.B. (2003). Recombinant adeno-associated virus vectors efficiently and persistently transduce chondrocytes in normal and osteoarthritic human articular cartilage. *Hum. Gene Ther.* 14, 393–402.
- Madry, H., Kaul, G., Cucchiari, M., Stein, U., Zurakowski, D., Remberger, K., Menger, M.D., Kohn, D., and Trippel, S.B. (2005). Enhanced repair of articular cartilage defects *in vivo* by transplanted chondrocytes overexpressing insulin-like growth factor I (IGF-I). *Gene Ther.* 12, 1171–1179.
- Marlovits, S., Kutscha-Lissberg, F., Aldrian, S., Resinger, C., Singer, P., Zeller, P., and Vecsei, V. (2004). Autologous chondrocyte transplantation for the treatment of articular cartilage defects in the knee joint: Techniques and results. *Radiologie* 44, 763–772.
- McCarty, D.M., Monahan, P.E., and Samulski, R.J. (2001). Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. *Gene Ther.* 8, 1248–1254.
- Nixon, A.J., Lust, G., and Vernier-Singer, M. (1992). Isolation, propagation and cryopreservation of equine articular chondrocytes. *Am. J. Vet. Res.* 53, 2364–2370.
- Rabinowitz, J.E., Rolling, F., Li, C., Conrath, H., Xiao, W., Xiao, X., and Samulski, R.J. (2002). Cross-packaging of a single adeno-associated virus (AAV) type 2 vector genome into multiple AAV serotypes enables transduction with broad specificity. *J. Virol.* 76, 791–801.
- Riviere, C., Danos, O., and Douar, A.M. (2006). Long-term expression and repeated administration of AAV type 1, 2 and 5 vectors in skeletal muscle of immunocompetent adult mice. *Gene Ther.* 13, 1300–1308.
- Sams, A.E., and Nixon, A.J. (1993). Studies of chondrocyte laden collagen scaffolds for resurfacing extensive equine articular cartilage defects. MS thesis, Cornell University, Ithaca, New York.
- Sanlioglu, S., Monick, M.M., Luleci, G., Hunninghake, G.W., and Engelhardt, J.F. (2001). Rate limiting steps of AAV transduction and implications for human gene therapy. *Curr. Gene Ther.* 1, 137–147.
- Saxer, R.A., Bent, S.J., Brower-Toland, B.D., Mi, Z., Robbins, P.D., Evans, C.H., and Nixon, A.J. (2001). Gene mediated insulin-like growth factor-I delivery to the synovium. *J. Orthop. Res.* 19, 759–767.
- Schmidt, M., Voutetakis, A., Afione, S., Zheng, C., Mandikian, D., and Chiorini, J.A. (2008). Adeno-associated virus type 12 (AAV12): A novel AAV serotype with sialic acid- and heparan sulfate proteoglycan-independent transduction activity. *J. Virol.* 82, 1399–1406.
- Ulrich-Vinther, M. (2007). Gene therapy methods in bone and joint disorders: Evaluation of the adeno-associated virus vector in experimental models of articular cartilage disorders, periprosthetic osteolysis and bone healing. *Acta Orthop. Suppl.* 78, 1–64.
- Ulrich-Vinther, M., Stengaard, C., Schwarz, E.M., Goldring, M.B., and Soballe, K. (2005). Adeno-associated vector mediated gene transfer of transforming growth factor- $\beta$ 1 to normal and osteoarthritic human chondrocytes stimulates cartilage anabolism. *Eur. Cell Mater.* 10, 40–50.
- Wu, Z., Asokan, A., and Samulski, R.J. (2006). Adeno-associated virus serotypes: Vector toolkit for human gene therapy. *Mol. Ther.* 14, 316–327.

Address correspondence to:  
 Dr. L.R. Goodrich  
 Orthopaedic Research Center  
 College of Veterinary Medicine  
 Colorado State University  
 Fort Collins, CO 80523

E-mail: laurie.goodrich@colostate.edu

Received for publication February 26, 2009;  
 accepted after revision July 29, 2009.

Published online: November 13, 2009.