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ARTICLE

Treatment of osteoarthritis using a helper-dependent adenoviral vector retargeted to chondrocytes

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Osteoarthritis (OA) is a joint disease characterized by degeneration of the articular cartilage, subchondral bone remodeling, and secondary inflammation. It is among the top three causes of chronic disability, and currently there are no treatment options to prevent disease progression. The localized nature of OA makes it an ideal candidate for gene and cell therapy. However, gene and cell therapy of OA is impeded by inefficient gene transduction of chondrocytes. In this study, we developed a broadly applicable system that retargets cell surface receptors by conjugating antibodies to the capsid of helper-dependent adenoviral vectors (HDVs). Specifically, we applied this system to retarget chondrocytes by conjugating an HDV to an α-10 integrin monoclonal antibody (a10mab). We show that a10mab-conjugated HDV (a10mabHDV)-infected chondrocytes efficiently *in vitro* and *in vivo* while detargeting other cell types. The therapeutic index of an intra-articular injection of 10mabHDV-expressing proteoglycan 4 (PRG4) into a murine model of post-traumatic OA was 10-fold higher than with standard HDV. Moreover, we show that PRG4 overexpression from articular, superficial zone chondrocytes is effective for chondroprotection in postinjury OA and that α-10 integrin is an effective protein for chondrocyte targeting.

INTRODUCTION

Osteoarthritis (OA) is a localized joint disease characterized by degeneration of articular cartilage, subchondral bone remodeling, and secondary intra-articular inflammation. It is a major cause of disability and one of the most common musculoskeletal disorders, costing the US health care system $100 billion annually.[1] Risk factors include mechanical stress, aging, and genetic predisposition.[2] Current treatments for OA are limited to lifestyle modifications, analgesics and nonsteroidal anti-inflammatory drugs, and in severe cases, joint replacement surgery. However, none of these treatments slow the progression of the disease.

In recent years, gene therapy has been clinically successful for localized diseases, especially genetic diseases that affect retinal function. Due to the localized nature of OA, gene therapy of the closed joint may also be successful without causing adverse effects such as the systemic immune response associated with intravascular delivery of adenovirus (AdV) vectors. Candidate genes for cartilage repair include inhibitors of catabolic factors as well as anabolic factors that promote chondrogenesis or maintenance of the chondrocyte phenotype. The former include interleukin-1 receptor antagonist (IL-1Ra), soluble tumor necrosis factor receptors, and tissue inhibitors of metalloproteinases.[3,4] The latter category includes the transforming growth factor (TGF-β) superfamily[2] and insulin-like growth factor (IGF)-1.[5] However, most growth factors induce fibrosis

and ectopic bone formation in laboratory animal models.[6] Moreover, these signaling pathways likely exert different effects on chondrocytes at various stages of differentiation and proliferation, and the long-term expression of these genes may cause opposing effects in a context- and temporal-dependent fashion. By contrast, our laboratory recently showed that Proteoglycan 4 (PRG4), a protein naturally secreted in synovial fluid, acts as an anabolic factor that slows the progression of OA in part by regulating the hypoxia-inducible factor transcriptional network in cartilage.[7] In the context of gene transfer in OA conditions, the rapid turnover rate of synoviocytes, which are efficiently and preferentially targeted by most viral vectors, reduces the expression of the therapeutic gene in the long term, thereby requiring higher doses with their concomitant dose-limiting toxicities.[8] Compared to synoviocytes, chondrocytes exhibit slower turnover in the context of OA especially in early stages of disease. Hence, they may be more effective and biologically relevant target for gene therapy in OA when using nonintegrating vectors.

AdV and adeno-associated vectors (AAV) are two of the most well-studied viral vectors *in vivo* for OA gene therapy.[8] We previously showed that, compared to AAV, helper-dependent adenoviral vectors (HDV) transduce chondrocytes at a higher efficiency.[7] In addition, the expression of genes transduced by HDV is sustained for more than a year when injected intra-articularly into a healthy mouse knee joint.[7] Despite that, one of the major obstacles

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in transducing cartilage specifically is that chondrocytes do not express the major receptor for AdV, the coxackie virus and adenovirus receptor (CAR).[9] This obstacle can be overcome and efficient transduction *in vivo* can still be achieved by requiring higher doses of AdV. Therefore, a receptor that facilitates effective transduction of chondrocyte would decrease potential toxicity in this particular context.

Integrins are α/β heterodimers that link the extracellular matrix with the cytoskeleton to mediate the activation of various signaling pathways.[10] In the joint capsule, α-10 integrin is expressed in chondrocytes as a surface receptor for collagen type II but not in synovia.[11,12] Genetically fusing the fiber capsid protein to the biotin acceptor peptide (BAP) of first-generation AdVs was one of several successful strategies used to retarget AdVs to cell types expressing specific membrane receptors.[13] However, this system has not been applied to HDVs, which show significant lower immunogenicity and long-term expression after a single injection.[14] Importantly, chondrocyte-specific targeting has not been achieved to date irrespective of gene delivery approach.

Here, we developed a novel HDV system by inserting the BAP into the fiber of the helper virus used for the preparation of the HDV. We show that the HDV with modified fiber, when conjugated with a monoclonal antibody against α-10 integrin (a10mab),[15] retargeted chondrocytes *in vitro* and *in vivo*. In addition, when we treated a murine model of post-traumatic OA with HDV retargeted to α-10 integrin (a10mabHDV) encoding PRG4 via intra-articular injection, we found that the effective treatment dosage was reduced 10-fold when compared to standard, untargeted, serotype 5 HDV. Hence, our data suggest that expression of PRG4 from superficial zone chondrocytes was sufficient to effectively delay the onset of post-traumatic OA. Together, our findings indicate a10mabHDV as a potential system for achieving long-term chondrocyte gene delivery and suggest that this vector may be further optimal for combinatorial gene expression given its high cloning capacity. Moreover, gene delivery of PRG4 using m10mabHDV was efficacious for the treatment of post-traumatic OA and showed improved therapeutic index compared to previous approaches.

RESULTS

Generation and characterization of a10mabHDV

We modified the helper virus used to generate HDV by genetically adding BAP to its fiber region (Figure 1a).[13,16] To test whether BAP mediates proper biotinylation in fiber-modified Ad (FM-Ad), we designed a series of enzyme-linked immunosorbent assay (ELISA) to assess the stepwise assembly of the vector-retargeting complex. We first assess biotinylation by coating the plate with either FM-Ad or serotype 5 adenovirus carrying LacZ (Ad-LacZ) and then detected a dose-related increase in biotin content using an anti-biotin antibody (Figure 1b). In assembling the retargeted vector, we generated and purified a10mabHDV by conjugating biotin, neutravidin (NA), and biotinylated a10mab in a stepwise manner (Supplementary Figure S1a–c). Again, we tested the level of biotinylation in HDV with the same method used for the helper virus. We observed an increase of biotin content proportional to increasing concentrations of the virus used to coat the wells (Figure 1c). Next, we tested the conjugation of NA by coating ELISA plates with NA-HDV. By detecting with a biotinylated primary antibody and horseradish peroxidase-conjugated secondary antibody, we observed a direct correlation between NA levels and virus concentrations (Figure 1d). Finally, to test whether a10mab is effectively conjugated to the vector particle, we coated the plate with a10mabHDV and detected its

presence with an anti-mouse secondary antibody against IgG. We observed a dose-dependent increase in absorbance (Figure 1e). In all three experiments, wild-type HDV-LacZ showed only baseline, background signals. Together, these data suggest that a10mabHDV properly assembled into a capsid and formed by an a10mab complex.

In vitro analysis of a10mabHDV transduction

To test whether a10mabHDV targets chondrocytes *in vitro*, we infected HEK293 cells and chondro-progenitor ATDC5 cells with a10mabHDV-LacZ. HEK293 cells express CAR but not α-10 integrin, while ATDC5 cells express a10 integrin but not CAR (Supplementary Figure S1d). Twenty-four hours after infection, we performed β-galactosidase staining to assess transduction efficiency. Wild-type HDV efficiently infected 293 cells but not ATDC5 cells; FM-HDV and NA-HDV infected both cell lines poorly. Compared to wild-type HDV, a10mabHDV transduced 293 cells less efficiently but infected ATDC5 cells more efficiently (Figure 2a). To quantify the changes in infection efficiency, we measured luminescence after cell lysis. As expected, we observed that a10mabHDV transduces ATDC5 cells about five times more efficiently than wild-type HDV while detargeting 293 cells (Figure 2b).

To test whether the increase in transduction efficiency of ATDC5 cells was mediated by a10mab, we performed a competitive inhibition assay by adding increasing concentrations of a10mab to the medium and measured cell luminescence 24 hours later. In 293 cells, no changes were observed with the addition of a10mab (Figure 2c), while in ATDC5 cells the infection efficiency decreased with increasing antibody concentrations (Figure 2d). These results suggest that a10mabHDV retargets chondro-progenitor cells and detargets other cell types *in vitro* via binding to a10 integrin.

In vivo analysis of a10mabHDV transduction

Although an increase of infection efficiency *in vitro* may decrease the required dose, and hence acute toxicity, effective treatment using AdVs will also require long-term gene expression *in vivo*. To investigate gene expression from a10mabHDV *in vivo*, we injected HDV and a10mabHDV expressing LacZ into the mouse knee joint. In healthy knee joints, wild-type HDV primarily transduced synoviocytes at a dose of 10^8 viral particles/joint. By contrast, a10mabHDV infected both synoviocytes and the superficial layer of chondrocytes at this dose (Figure 3a, Supplementary Figure S1e). We observed similar results in a knee joint with OA induced by cruciate ligament transection (CLT).[17] Interestingly, the number of cells infected in OA knee joints was greater than in uninjured knees, likely due to the exposure of articular chondrocytes to the vector in the OA model (Figure 3a).

Since OA is a chronic disease, gene transfer approaches to treatment need to exhibit long duration of expression. Alternatively, repeated injections of virus could be pursued but this may lead to an increased host immune response. Our published data show that after intra-articular injection into the mouse knee joint, HDV can mediate sustained gene expression for more than one year *in vivo*.[7] Therefore, we assessed whether a10mabHDV could also mediate long-term gene expression and followed the expression of luciferase by *in vivo* imaging up to three months after intra-articular injection of wild-type HDV or a10mabHDV carrying luciferase at 10^9 viral particle/joint. With either HDV-luciferase or a10mabHDV-luciferase injection, luciferase expression was sustained for at least 3 months, while first-generation AdVs lose expression after 3 weeks. Luciferase transduced by a10mabHDV was expressed at lower levels
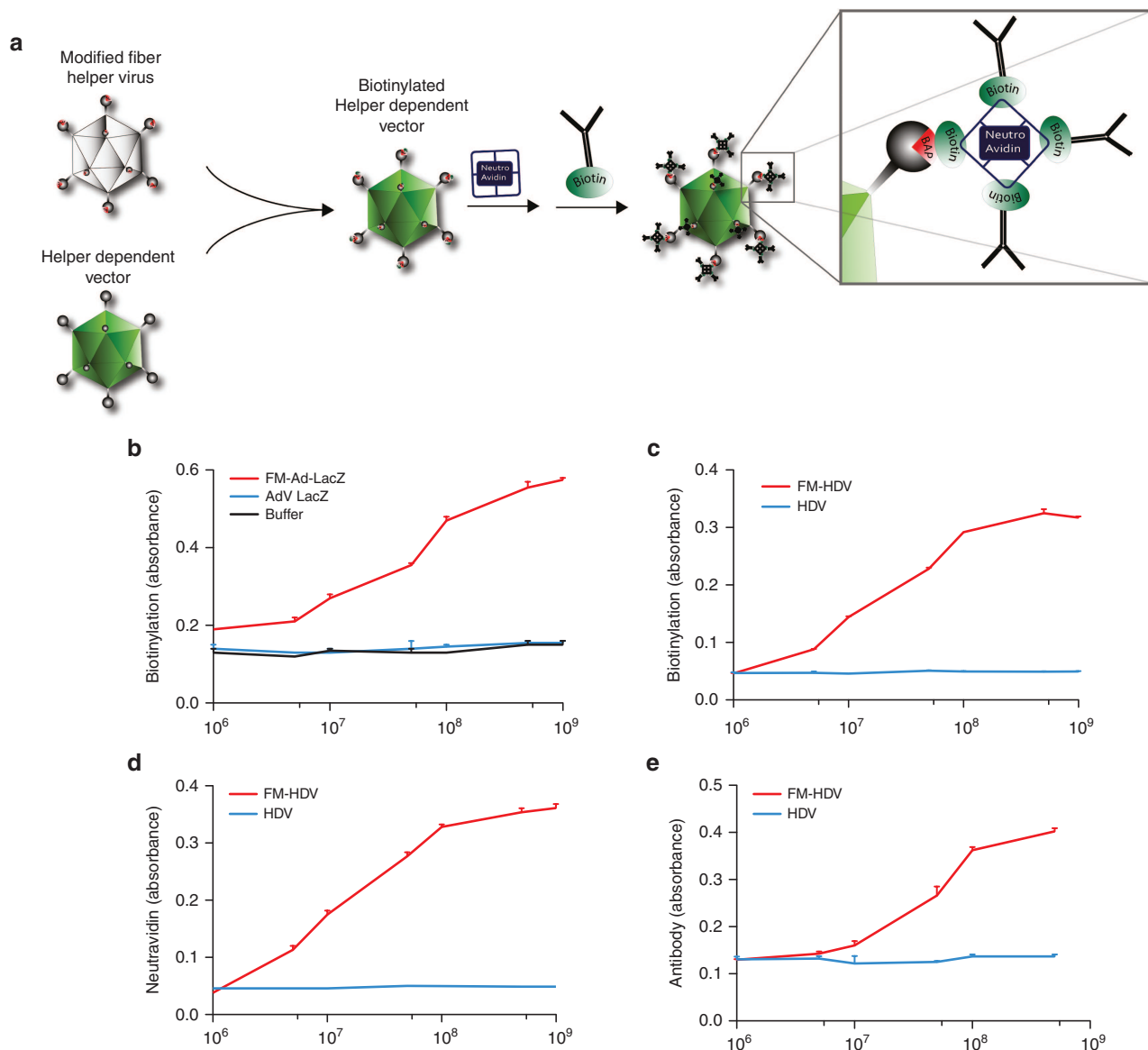
Figure 1 Assessment of conjugation of helper virus to a10mabHDV by enzyme-linked immunosorbent assay. (**a**) Biotin acceptor peptide sequence was inserted onto the HI-loop of the fiber of the helper virus. This fiber-modified virus can be used to rescue a helper dependent adenoviral vector. Subsequently, the biotinylated HDV can be complexed with NeutrAvidin (NA) and then with a biotinylated antibody for retargeting of the vector. (**b**) Amount of biotin in biotinylated fiber-modified helper virus (FMAdV-LacZ) and wild-type helper virus (AdV-LacZ) at different concentrations. Absorbance of FM-Ad-LacZ is significantly different from AdV LacZ and buffer statistically. (**c**) Amount of conjugated biotin in biotinylated fiber-modified HDV (FM-HDV) and serotype 5 HDV (HDV) at different concentrations. (**d**) Amount of conjugated neutravidin in fiber-modified HDV (FM-HDV) and serotype 5 HDV (HDV) at different concentrations after conjugation of neutravidin. (**e**) Amount of conjugated α-10 integrin antibody in fiber-modified HDV (FM-HDV) and serotype 5 HDV (HDV) at different concentrations. (**c–e**) Absorbance of FM-HDV is significantly different from HDV statistically. All experiments included four technical repeats. Error bar indicates SD.

compared to unmodified HDV, which was likely due to detargeting of synoviocytes (Figure 3b).

Prevention of OA by low-dose a10mabHDV

Prg4 has been previously established as a candidate gene to treat osteoarthritis.[18,19] Our studies showed that intra-articular injection of wild-type HDV-Prg4 prevented post-traumatic OA in the mouse.[7] However, when HDV is injected at a dose of 10^8 viral particle/joint, HDV only transduces synoviocytes. Since PRG4 is naturally expressed in the superficial layer of chondrocytes,[20] chondrocyte transduction of PRG4 may be more effective in preventing OA than synoviocyte transduction. To test this hypothesis,

we injected a10mabHDV-PRG4 at the lower 10^8 viral particle/joint dose. Twenty-four hours after injection, we performed CLT to induce post-traumatic OA.[21] At 1 and 2 months later, we collected joint samples and assessed development of OA by histology and phase contrast micro-computed tomography (microCT) quantification.[21,22] Results collected 1 month after injection (Figure 4a–c) and 2 months after injection (Figure 4d–f) showed similar trends, while the beneficial effects of a10mabHDV were more prominent in the long-term experiment. A lower score in the histological scoring scale of articular cartilage injury of the OA Research Society International (OARSI) indicates less severe OA. Both HDV-PRG4 and a10mabHDV-PRG4 treatment prevented OA development at
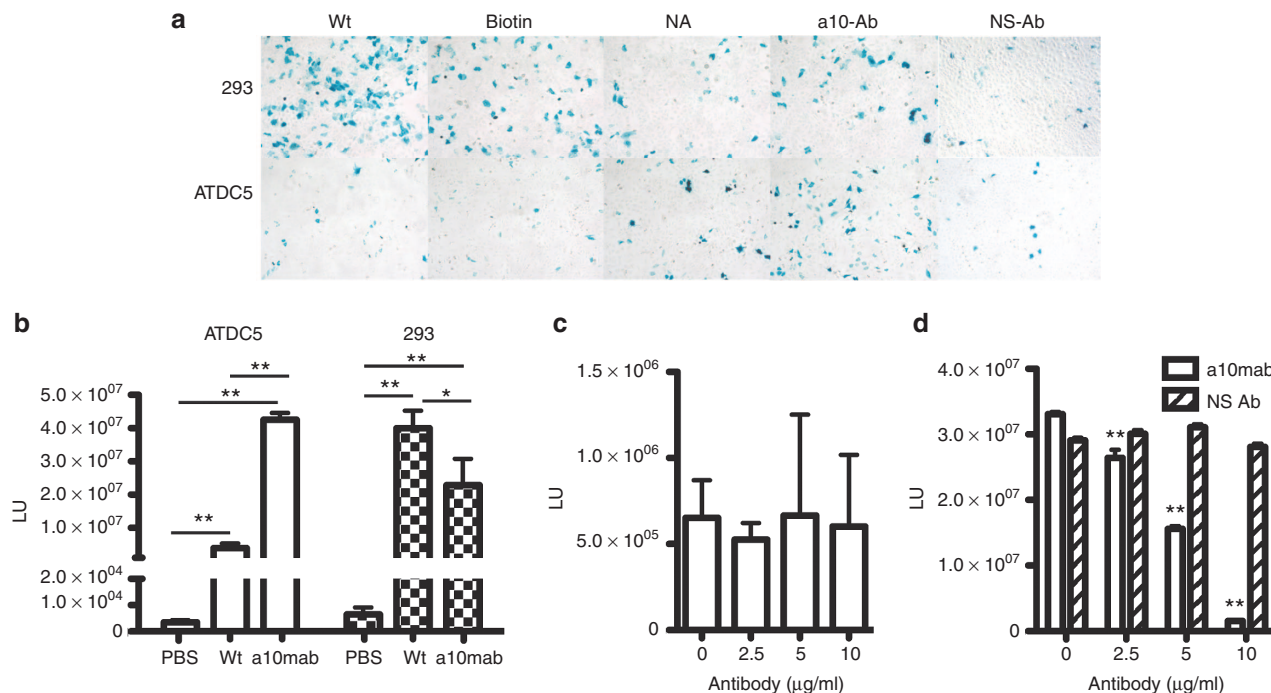
Figure 2 *In vitro* characterization of a10mabHDV. (**a**) β-galactosidase staining of 293 and ATDC5 cells 24 hours after infection of serotype 5 helper-dependent virus (Wt), FM-HDV conjugated with biotin (Biotin) or neutravidin (NA), and a10mabHDV (a10-Ab) carrying LacZ. FM-HDV conjugated with nonspecific mouse IgG carrying LacZ (NS-Ab) served as negative control. (**b**) Luminescence of β-gal level 24 hours after infection of HDV and a10mabHDV in ATDC5 cells and 293 cells. $N = 4$, **$P < 0.01$; error bar indicates SD. (**c**) Luminescence of β-galactosidase expression in 293 cells 24 hours after infection of a10mabHDV in media containing a10mab. $N = 4$; error bar indicates SD. (**d**) Luminescence of β-galactosidase level in ATDC5 cells 24 hours after infection of a10mabHDV in media containing a10mab and nonspecific antibody (NS Ab). $N = 4$, *$P < 0.05$, **$P < 0.01$; error bar indicates SD.

all doses tested, although 10mabHDV-PRG4-treated mice exhibited a lower mean score, suggesting greater efficacy (Figure 4a,d). Phase contrast microCT quantification showed that both HDV-PRG4 and a10mabHDV-PRG4 preserved cartilage volume and surface area. Importantly, the injection of a10mabHDV-PRG4 at 10^8 viral particle/joint achieved similar results as HDV-PRG4 at the higher 10^9 viral particle/joint (Figure 4b,c,e,f), supporting at least a 10-fold improvement in the therapeutic index attributable to the retargeting the vector to superficial zone chondrocytes.

Treatment of OA with a10mabHDV

With the development of OA and secondary inflammation, transduction of synoviocytes by HDV may be reduced rapidly due to synoviocyte turnover and proliferation. In our previous studies, a dose of 10^8 viral particle/joint of HDV-PRG4 started to lose its effectivenss 2 months after CTL.[7] Since a10mabHDV transduces chondrocytes, we tested whether a10mabHDV-PRG4 can treat post-traumatic OA at the same low dosage. We injected a10mabHDV-PRG4 at 10^8 viral particle/joint 2 weeks after CLT, simulating the delayed treatment likely to occur in clinical scenarios. We then assessed the development of OA 2 months after CLT by histological scoring and phase-contrast microCT. In contrast to HDV-PRG4 injection, we observed a positive treatment effect with a10mabHDV-PRG4 injection by OARSI scoring (Figure 5a). In addition, we observed greater preservation of cartilage volume with a10mabHDV-PRG4 injection compared to HDV-PRG4 injection at the same dose. Finally, knee joints injected with a10mab-HDV-PRG4 showed a larger bone area covered by cartilage compared to the ones injected with HDV-PRG4 at the same dosage (Figure 5b,c).

DISCUSSION

The development of targeted vectors has been a focus in gene therapy because the transduction of specific cells reduces the required dosage while enhancing treatment efficacy with reduced toxicity. Here, we describe a vector created by genetic modification of a HDV followed by chemical conjugation of chondrocyte-specific a10mab monoclonal antibody. The conjugated virus, a10mabHDV, targets chondrocytes both *in vitro* and *in vivo*. Compared with the untargeted HDV-PRG4, a10mabHDV-PRG4 reduced the effective dosage requirement for preventing post-CTL OA 10-fold.

Both cell and gene therapies have been tested in the treatment of OA. In two clinical trials, retrovirus was studied as a vector for *ex vivo* OA therapy.[23] Both trials utilized chondrocyte cells transduced with TGF-β1-expressing retrovirus; cells were irradiated before intra-articular injection to avoid potential oncogenic activities. No serious adverse effects were reported in the phase 1 of these clinical trial.[24] However, genes introduced by retroviral vectors were not expressed at high level due to low copy number integration of the vector. Most *in vivo* OA gene therapy trials to date have used AAV, with a phase 1 clinical trial directly delivering interleukin-1 receptor antagonist (IL1Ra).[8,23] Wild-type AAV does not cause any known disease and is thought to exhibit lower immunogenicity,[25] but despite its ability to transduce chondrocytes and synoviocytes it did not show long-term transduction in immune-competent animals.[26] In addition, the packaging capacity of AAV is 5 kb, limiting the possibility of transducing large genes like Prg4 and of combinatorial gene therapy with other transgenes.

Our previous data suggest that, in the short-term, HDV serotype 5 mediates higher transduction efficiency compared to the three most
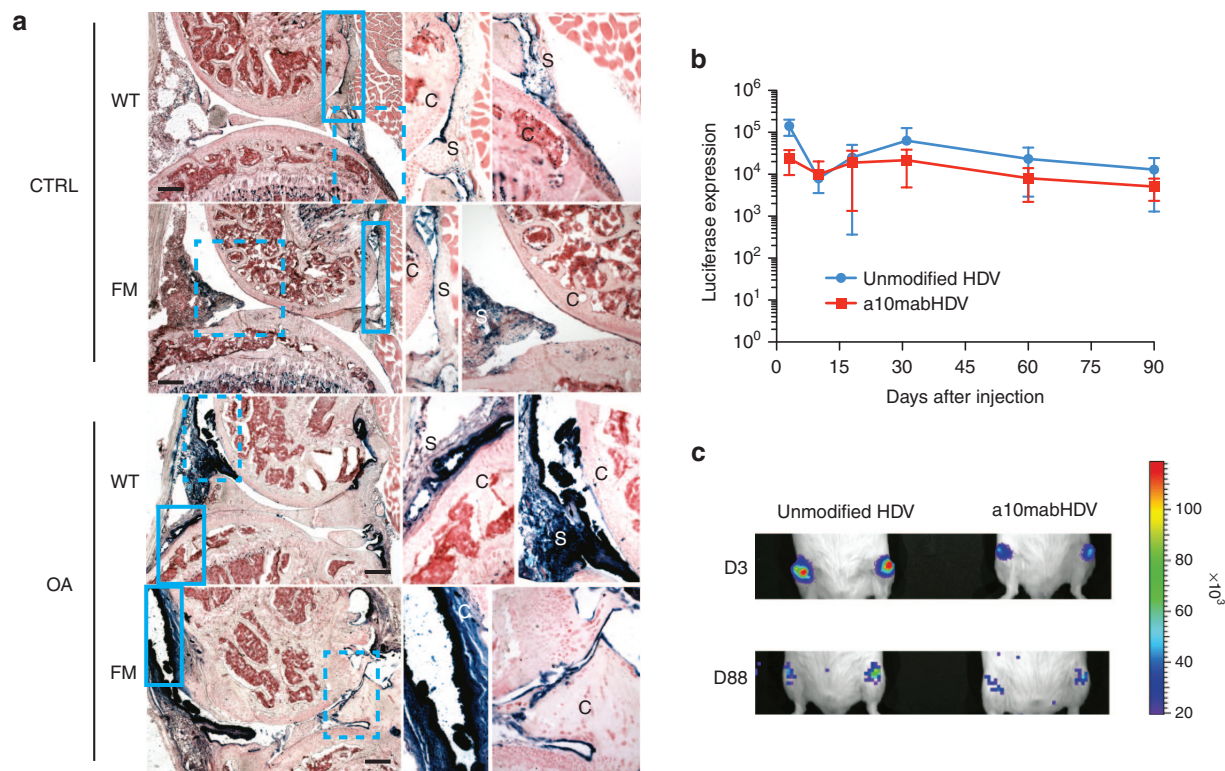
Figure 3 *In vivo* characterization of a10mabHDV. (**a**) β-galactosidase staining of healthy and osteoarthritic knee joint 2 weeks after intra-articular injection of WT-HDV and a10mabHDV. Images on the left show whole knee joints; images in the middle and on the right are enlarged images of the boxed area with solid line and dotted line, respectively. S: Synovia, C: cartilage. Scale bar, 200 μm. (**b**) Longitudinal expression in knee joint after intra-articular injection of WT-HDV and a10mabHDV measured by luciferase. Error bars represent SD, $N = 10$. (**c**) Representative images of mouse knee joints from luciferase imaging from day 3 after injection and day 88 after injection.

popular AAV serotypes used in OA, serotype 2, 2.5, and 6, at equivalent dosing.[7] Due to its lower immunogenicity compared to first-generation AdV, HDV can achieve long-term expression, up to 1 year after a single intra-articular injection in mice.[7] With the genetic modification of virus fiber followed by a10mab conjugation, HDV was retargeted to chondrocytes enabling an efficacious dosing, with a dose approximately 100 times lower than the maximal tolerated dose in humans.[27] However, the immune reactions of one species might not predict those of another species, and thus large animal studies are necessary to further validate this approach prior to clinical evaluation.

We used PRG4 as loss of its function in humans causes early onset OA, whereas gain of function in mice protected from both age-related OA changes and post-CLT OA. PRG4 confers protection mice from OA development by decreasing physical friction and inhibiting the cartilage catabolic pathways.[7] By contrast, other gene therapy approaches for OA have targeted cartilage growth factors (*e.g.*, IGF1), transcriptional factors (*e.g.*, TGF-β1), inhibitors of matrix degrading enzymes (*e.g.*, ADAMTS5), and anti-inflammatory factors (IL1Ra).[28] Clinical trials featured recombinant proteins including antibodies against tumor necrosis factor, IL1R, bone morphogenic protein 7 (BMP7), and fibroblast growth factor 18 (FGF 18).[29] Most of these studies are still ongoing. Among these recombinant proteins, IL1Ra was the only one that demonstrated positive results. Patients with a single intraarticular injection of IL1Ra reported less pain according to the standardized Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire at 14 days after injection.[30] The results of these clinical trials can be contributed to the following reasons. Firstly, cartilage growth factors and transcriptional factors usually mediate cartilage growth and repair with good short-term results, but they cause significant long-term

adverse effects, including calcification of repaired cartilage and accelerated bone remodeling. These effects might reflect the often temporal and spatial restriction of transcription factors during the various stages of cartilage development. However, with better technologies to control the timing of expression, these factors may be used for cartilage regeneration *in vivo*. Secondly, inhibitors of matrix degrading enzymes protect animals from OA by blocking cartilage degeneration, but many of these enzymes are necessary for the physiological metabolism of cartilage and bone and inhibiting them may alter the normal metabolic processes in these tissues. Finally, inflammation is usually secondary in OA. Therefore, anti-inflammatory genes might not prevent early stage OA and cartilage degradation, although they may be important candidates in combinatorial approaches. A HDV can carry up to 32 kb of DNA and is optimal for delivery of regulatory switches and combinations of therapies targeting different aspects of the disease.

In summary, we developed a retargeted HDV vector expressing PRG4 (a10mabHDV-PRG4) as a novel treatment modality for OA. Transduction of superficial layer zone chondrocytes effectively reduced the required dose at least 10-fold compared to the untargeted vector. Moreover, our system sets the stage for a platform that can combine the expression of multiple genes targeted at the complex pathophysiology of OA in the context of localized gene therapy.

MATERIALS AND METHODS

Adenoviral vector generation

Wild-type serotype 5 HDV vectors were generated as previously described.[31] To obtain a10mabHDV, helper virus was generated by cloning the BAP fragment to the knob region of the fiber. Cells were cultured in Corning
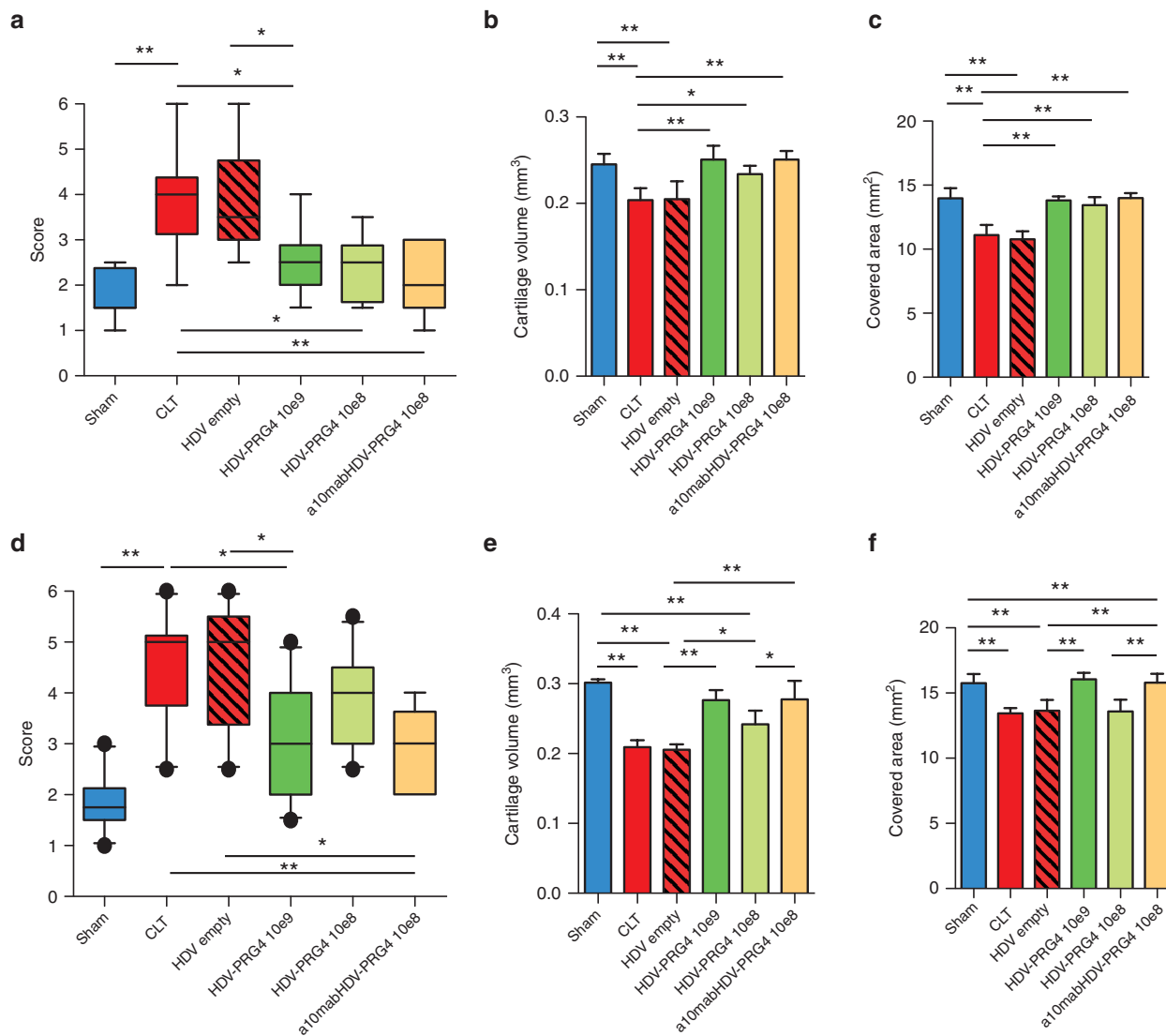
Figure 4 Prevention of OA by WT-HDV and a10mabHDV expressing PRG4. (**a–c**) Two-month-old mice were injected with different types of viruses (or no injection). Twenty-four hours after injection, CLT or sham surgeries were performed. Histology scoring (**a**), cartilage volume (**b**), and cartilage covered bone area (**c**) were measured 1 month after CLT. (**d–f**) Two-month-old mice were injected with different types of viruses (or no injection). Twenty-four hours after injection, CLT or sham surgeries were performed. Histology scoring (**d**), cartilage volume (**e**), and cartilage covered bone area (**f**) were measured 2 months after CLT. Sham, sham surgery at 2 months old; CLT, cruciate ligament transection surgery at 2 months old. HDV empty, Empty HDV injection at 10^9 vp/joint followed by CLT 24 hours later. HDV-PRG4 10^9, HDV-PRG4 injection at 10^9 vp/joint followed by CLT 24 hours later. HDV-PRG4 10^8, HDV-PRG4 injection at 10^8 vp/joint followed by CLT 24 hours later. a10mabHDV-PRG4 10^8, a10mabHDV-PRG4 injection at 10^8 vp/joint followed by CLT 24 hours later. *$P < 0.05$, **$P < 0.01$; error bars represent SD. $N = 10$ for histological scoring, $N = 5$ for cartilage volume and surface area measurement.

10-chamber cellSTACK cell culture chamber (Corning, Corning, NY) with 1 g biotin/l media. After the first 1.25 g/1.35 g CsCl gradient purification, viral bands were removed from the tube and transferred to a 15 ml tube. The volume of the virus was brought up to 4 ml with 100 mmol/l Tris A separate tube with 4 ml NA-Tris solution was prepared (100 µg/ml). Virus solution was added drop by drop to the NA-Tris solution, keeping the NA-Tris solution on a low speed vortexer. After a 30-minute incubation in dark on ice, the mix was loaded onto another 1.25 g/1.35 g CsCl gradient and centrifuged at 150,000 g for 2 hours at 4 °C. Next, the virus band was retrieved again and transferred to a new 15 ml Falcon tube, with a total volume of 4 ml in 100 mmol/l Tris. The virus solution was added drop by drop to the antibody-Tris solution, providing a 10× excess of antibody. After a 30-minute incubation in the dark on ice, the mixture was loaded onto a 1.35 g/1.5 g CsCl gradient and centrifuged at 150,000 g at 4 °C overnight. The purified virus was retrieved the next day and put into a dialysis cassette presoaked in dialysis buffer (10 mmol/l Tris-Cl, pH 8) at 4 °C. The virus was retrieved after changing the dialysis buffer twice and stored at −80 °C after addition of 10% glycerol.

ELISA

The proper conjugations of a10mabHDV were characterized by ELISA designed in the laboratory. After virus dilution, Immulon 2HB 96-well U-bottom plates (VWR) were coated with virus diluted with coating buffer (0.1M $NaHCO_3$-$NaCO_3$, pH 9.5) at 100 µL/well. After incubating for 1.5 hours at 37 °C, plates were blocked with 5% milk powder in TBS-Tween for 2 hours at 37 °C. Then, the plates were incubated with primary antibody, at the proper dilution at 100 µL/well for 2 hours at 37 °C. After this, plates were incubated with secondary antibody at the proper dilution at 37 °C for 2 hours. Plates were developed with 100 µL developing buffer/well for 3 minutes, and the reaction was stopped with 50 µL 2N H_2SO_4. Absorbances were measured at 450 nm. To prepare the developing buffer, one tablet of 3,3',5,5'-Tetramethylbenzidine dihydrochloride tablets (Sigma) and 3 µL of 30% hydrogen peroxide were added to 10 ml of citrate/phosphate buffer (citric acid 1.92 g/100 ml, $NaHPO_4$ 2.84 g/100 ml, pH5). TBS-Tween (50 mmol/l Tris, 100 mmol/l NaCl, 0.05% Tween) was used to wash the plates four times between incubations and before development.
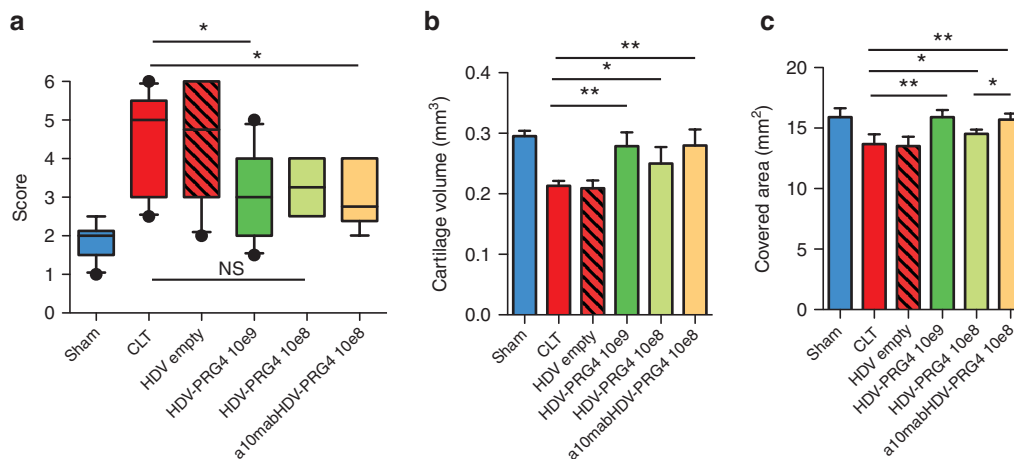
Figure 5 Treatment of OA by wt-HDV and a10mabHDV expressing PRG4. Two-month-old mice underwent sham or CLT. Two weeks after the CLT, different treatments were performed. Samples were collected 2 months after CLT. (**a**) Histological scoring of groups with different treatments. (**b,c**) Cartilage volume (**b**) and cartilage covered bone area (**c**) for the different treatment groups. Sham, sham surgery at 2 months old. CLT, cruciate ligament transection surgery at 2 months old. HDV empty, Empty HDV injection at 10^9 vp/joint 2 weeks after CLT. HDV-PRG4 10^9, HDV-PRG4 injection at 10^9 vp/joint 2 weeks after CLT. HDV-PRG4 10^8, HDV-PRG4 injection at 10^8 vp/joint 2 weeks after CLT. a10mabHDV-PRG4 10^8, a10mabHDV-PRG4 injection at 10^8 vp/joint 2 weeks after CLT.*$P < 0.05$, **$P < 0.01$; error bars represent SD. $N = 10$ for histological scoring, $N = 5$ for cartilage volume and surface area measurement.

Cell culture and *in vitro* infection

293 cells were maintained in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum; ATDC5 cells were maintained in DMEM/F-12 1:1 mixture supplemented with 10% FBS. In the *in vitro* infection analysis, cells were plated 24 hours before the infection in order to reach 85% confluency at the time of infection. Both WT and a10mab HDV were diluted at 100 viral particle/cell in phosphate-buffered saline (PBS) with magnesium and calcium (Hyclone) in a sufficient volume to cover the cells (*e.g.*, 100 μl/well in a six-well plate). The medium was aspirated from the cells and replaced with virus solutions. After 1 hour of incubation, virus solutions were aspirated and fresh medium was added. Both ATDC5 and 293 cells were incubated with different concentrations of antibody against a10 integrin (Xintela AB) dissolved in PBS before being analyzed on a FACS caliber flow cytometer (BD Biosciences). The results were analyzed with the FlowJo software (Tree Star).

Luminescence measurement

Luminescence of β-galactosidase was measured with the Luminescent Beta-galactosidase Detection Kit (CLONTECH) according to the manufacturer's protocol.

Animals

FVB/N mice were purchased from Jackson Laboratories (Bar Harbor, ME). This strain was used because of its large litter size. All studies were performed with approval from the Baylor College of Medicine Institutional Animal Care and Use Committee (IACUC). Mice were housed under pathogen-free conditions and had free access to food and water at all times.

CLT surgery

CLT and sham surgeries were performed as previously described in 8-week-old male FVB/N mice.[21]

HDV injection

Mice were anesthetized using 3% isoflurane, and the joint area was shaved. HDVs were diluted in 5 μl of sterile PBS and injected with 25 μl CASTIGHT syringes (1702 Hamilton Company) and 33-gauge needles (7803–05 Hamilton Company) from the medial side of the knee joint.

Histology

Mice were euthanized and samples were fixed with 4% paraformaldehyde (Sigma-Aldrich) overnight at 4 °C on a shaker. For safranin O staining, samples were decalcified in 14% EDTA for 7 days at 4 °C on a shaker. Paraffin embedding was performed using standard protocol. Samples were sectioned at 6 μm and stained with safranin O and Fast Green using standard protocols. Samples were scored by two independent pathologists masked to the procedure. For LacZ staining, samples were decalcified in 14% EDTA for 3 days at 4 °C on a shaker. After washing with PBS three times, samples were then embedded in optimal cutting temperature compound (Tissue-Tek) and frozen-sectioned at 6 μm with the Cryojane tape transfer system (Leica Microsystems). Samples were subsequently stained with X-gal (X428IC Gold Biotechnology) overnight using Nuclear Fast Red (N3020 Sigma) as counterstain. All the stainings in the same experiment were done at the same time.

Luciferase assay

Mice were injected with 2 mg D-luciferin (L9504 SIGMA) diluted in 100 μl PBS per mouse (25 g) intraperitoneally and anesthetized using 2.5–3% isoflurane. Images were collected 15 minutes after luciferin injection by Xenogen IVIS optical *in vivo* imaging system. Quantification was performed with Living Imaging 4.2 using default settings. Values were normalized to control mice.

Phase-contrast microCT imaging and analysis

Samples were prepared as previously described, scanned by Xradia μXCT and analyzed using the TriBON software (RATOC, Tokyo, Japan), as previously described.[21] Observers were blinded to the procedure and sample number.

Statistics

Statistical significance comparing two groups with parametric data was assessed by Student's *t*-test. Statistical analysis comparing multiple groups with parametric data was performed by one-way analysis of variance followed by Tukey's *post-hoc*. Normality was tested by Shapiro-Wilk Normality test, and histological grades were compared by Wilcox rank test. All analyses were performed with the SPSS software or Sigma Plot. A *P* value of <0.05 was considered statistically significant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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