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Cyclic-RGD Peptides Increase the Adenoviral Transduction of Human Mesenchymal Stem Cells

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Human mesenchymal stem cells (hMSCs) have been extensively explored for drug delivery applications due to their safety, immunomodulatory properties, and ability to differentiate into new tissues. The experiments presented in this study were designed to determine peptide-based mechanisms to increase the adenoviral transduction of hMSCs for the purpose of improving their capacity as drug delivery vehicles. Specifically, we demonstrated that cyclic- RGD peptides increased the internalization of adenoviruses into MSCs. MSCs treated with cyclic-RGD peptides had a transduction efficiency of 76.6% ± 4%, which was significantly greater than the 23.5% ± 12.2% transduction efficiency of untreated stem cells ($P < 0.05$). Blocking endocytosis with inhibitors of dynamin or actin polymerization decreased the cyclic-RGD-mediated increase in transduction efficiency. MSCs treated with cyclic-RGD and adenoviruses carrying the gene for bone morphogenetic protein-2 produced significantly greater concentrations of this growth factor compared to stem cells treated with only adenoviruses or adenoviruses cocultured with cyclic-RAD peptides. Furthermore, this stem cell-produced bone morphogenetic protein induced alkaline phosphatase expression in C2C12 cells indicating growth factor bioactivity. Taken together, these studies suggest that cyclic-RGD peptides could be used to increase the adenoviral transduction of hMSCs and increase their therapeutic potential.

Introduction

Several studies have demonstrated the capacity of human mesenchymal stem cells (hMSCs) to differentiate into functional adult cell types as well as their ability to produce therapeutic extracellular factors. The National Institutes of Health currently lists 129 clinical trials using autologous and allogeneic hMSCs for diverse indications (http://clinicaltrials.gov). To date, results from these trials have indicated that hMSCs generally produce temporary improvements that may be primarily attributed to their production of soluble signaling molecules [1]. hMSCs have several advantageous properties for drug delivery applications, including their ability to be expanded in culture without losing their phenotype, their immunoprivileged nature, innate immunomodulatory properties, their ability to produce diverse extracellular factors, and their potential to home to damaged tissue sites [2]. Therefore, hMSCs have been studied as drug delivery vehicles for growth factors [3–5], proteases [6], oncolytic adenoviruses [7], transcription factors [8], and chemokine receptors [9]. In each of these applications, increasing the capacity of hMSCs to produce extracellular factors is critical for their therapeutic success.

An emerging approach to enhance the therapeutic effectiveness of transplanted hMSCs has been to transduce them with therapeutic adenoviruses. hMSCs have been transduced with viruses containing the genes for diverse proteins, including the coagulation factor IX [6], the bone morphogenetic protein-2 (*BMP2*) [10], the vascular endothelial growth factor [11], interferon-β [12], and leptin [13]. For example, hMSCs transduced with adenoviruses carrying the gene for insulin-like growth factor-1 homed to ex-vivo cultured bovine intervertebral disks and induced greater proteoglycan synthesis compared to unmodified hMSCs [14]. Taken together, these results suggest that new methods to increase the internalization of therapeutic viruses into hMSCs could be broadly applied to increase their clinical efficacy.

One potential mechanism to increase the adenoviral transduction of hMSCs would be to harness their endogenous internalization mechanisms using integrin-binding peptides. Adenoviruses have been characterized during cell entry to first bind to their primary coxsackie and adenovirus receptors [15]. After docking with their primary receptors, adenoviruses bind to integrins via their surface-exposed RGD peptide sequence. This integrin binding initiates intracellular processes, including endocytosis [16]. Critically, integrin binding has not been necessary for adenoviral internalization [17]. In fact, linear GRGDSP peptides have inhibited the adenoviral transduction of HeLa and M21 cells.

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Therefore, we sought to determine the effect of RGD peptide sequence and structure on the transduction efficiency of hMSCs.

In this study, we characterized the ability of RGD-based peptides to increase the adenoviral transduction efficiency of hMSCs and determined the extent to which the increased transduction efficiency would translate to enhanced production of therapeutic proteins. hMSCs from bone marrow were chosen because they possess increased genetic stability compared to hMSCs from other tissues [18]. RGD-based peptides were chosen because of their well-characterized role in integrin signaling, cell adhesion, biomaterials design, and approval for use as an orphan drug by the European Medicines Agency and the United States Food and Drug Administration [19]. Therapeutic adenoviruses were characterized in detail because they have been used to insert genes into hMSCs [20,21] with greater efficiency than nonviral transfection techniques [22]. Results demonstrated that cyclic-RGD molecules enhanced the uptake of therapeutic viruses. Cyclic-RGD peptides may have increased the internalization of adenoviruses through the endocytosis pathway. The specific peptides increased the concentration of an important therapeutic protein, BMP2, after transduction with adenoviruses carrying the gene for *BMP2*. Significantly, hMSC-produced BMP2 was bioactive and induced increased alkaline phosphatase gene expression in C2C12 cells, which have been the canonical test for BMP2 bioactivity [23]. These studies suggest that cyclic-RGD peptides could be broadly employed to increase the drug delivery potential of hMSCs for diverse therapeutic indications.

Materials and Methods

Stem cell culture

hMSCs were purchased from Lonza (Walkersville, MD) and grown in the α-MEM medium (Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum (Gibco), and 1% penicillin/streptomycin (Gibco). The cells were grown following protocols to maintain their multipotency [24]. Twenty-four hours before transduction, hMSCs were passaged into 24-well plates (Corning, Corning, NY) at a seeding density of 1.3×10^4 hMSCs/cm^2. Cyclic-RGD (RGDfV) (capital letters = L amino acids, lower case letters = D amino acids), Cyclic-RAD (RADfV) (Peptides International, Louisville, KY), and linear-RGD peptides (GRGDSP) (Anaspec, Fremont, CA) were diluted in phosphate-buffered saline (PBS) (Gibco) and stored at −20°C until treatment. hMSCs were imaged using an Eclipse TE2000-s (Nikon, Melville, NY) inverted microscope controlled using NIS Elements D 3.1 software (Nikon).

Macromolecule internalization techniques

hMSCs were passaged into 24-well plates as previously described to examine the effects of peptides on adenovirus uptake. To quantify adenovirus internalization, hMSCs were treated with predetermined cyclic-RGD concentrations and adenoviruses carrying the gene for *BMP2* (Ad-BMP2) (University of Michigan Vector Core, Ann Arbor, MI) at a multiplicity of infection (MOI) of 250 for 24 h. After the incubation period, hMSCs were washed 4 times with 4°C PBS and fixed using Z-Fix (Anatech, Battle Creek, MI). The cells were then treated with a mouse anti-adenovirus primary antibody and a fluorescein isothiocyanate-labeled goat anti-mouse secondary antibody (Abcam, Cambridge, MA). Bright field and fluorescent micrographs were collected and greater than 10 micrographs per treatment were quantified by normalizing the number of fluorescent cells to the total number of cells per micrograph.

Transduction methods

Adenoviruses carrying the genes for the green fluorescent protein (Ad-*GFP*) and Ad-*BMP2* were acquired from the University of Michigan Vector Core and diluted to their final MOI using PBS (Gibco) containing 0.5% gelatin (Fisher Scientific, Fairlawn, NJ). hMSCs were washed with PBS, and then the diluted adenovirus was added in 0.25 mL serum containing the medium for 24 h. Then, the adenovirus containing media was replaced with fresh hMSC media. The transduction efficiency was measured using 2 techniques. First, bright field and fluorescent micrographs of Ad-GFP-transduced hMSCs were collected from 4 wells in a 24-well plate and at least 10 micrographs were quantified per treatment. To qualify this image-based analysis, hMSCs were treated using the same procedure, and then their normalized transduction efficiency was analyzed using a C6 Flow Cytometer (BD Accuri Cytometers, Ann Arbor, MI).

Characterization of internalization pathways

The effect of cyclic-RGD peptides on endocytosis was studied using pharmacological inhibitors that blocked endocytosis. hMSCs in 24-well plates were treated for 30 min with 80 μM Dynasore (Santa Cruz Biotechnology, Santa Cruz, CA) or 5 μM cytochalasin B (Fisher Scientific). The cells were then washed with PBS and fresh hMSC media was added with predetermined concentrations of cyclic-RGD and Ad-GFP for 24 h. The hMSC transduction efficiency was then measured using the previously described image-based quantification techniques. To determine if these concentrations were effective at blocking endocytosis, hMSCs were treated with 1 mg/mL Lucifer Yellow for 24 h (Fisher Scientific). hMSCs were then washed 4 times with 4°C PBS, fixed using Z-Fix, and imaged using previously described techniques.

Characterization of therapeutic relevance

To demonstrate the therapeutic relevance of this peptide-based approach, hMSCs were tested as a drug delivery vehicle for BMP2. First, hMSCs were treated with Ad-BMP2, Ad-BMP2 + cyclic-RGD, Ad-BMP-2 + cyclic-RAD, Ad-GFP, cyclic-RGD or they were maintained in the hMSC growth medium for 24 h. The medium was collected and replaced after 4 and 7 days. All experiments were performed in 0.25 mL hMSC medium in 24-well plates ($n = 6$). The BMP2 concentration in the medium produced by hMSCs was quantified using an enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's protocol (R&D Systems, Minneapolis, MN).

C2C12 cells (ATCC, Manassas, VA), which upregulate alkaline phosphatase expression in response to BMP2, were used to evaluate the bioactivity of the hMSC-produced BMP2. C2C12 cells were grown in the DMEM (Gibco) with

10% fetal bovine serum (Gibco), and 1% penicillin/streptomycin (Gibco). C2C12 cells were passaged into 24-well plates at a seeding density of 1.3×10^4 cells/cm^2 and grown for 24 h. The cells were then washed with PBS and grown in a conditioned medium from the hMSCs treated with Ad-BMP2, Ad-BMP2+ cyclic-RGD, Ad-BMP-2+ cyclic-RAD, Ad-GFP, cyclic-RGD, or media from hMSCs that were left untreated. The C2C12 cells were maintained in this conditioned medium for 3 days, and then stained for alkaline phosphatase activity using a Leukocyte Alkaline Phosphatase Kit following the manufacturer's instructions (Sigma, St. Louis, MO). All experimental conditions were performed in quadruplicate and repeated at least 5 times. The effect of cyclic-RGD peptides alone on alkaline phosphatase activity in C2C12 cells was tested by culturing them in increasing concentrations of the peptide for 24 h. The medium was then replaced with a fresh C2C12 medium; the cells were cultured for an additional 3 days and finally stained for alkaline phosphatase activity. Greater than 15 bright field micrographs were collected per treatment and the number of alkaline phosphatase expressing cells and the total number of cells were quantified using previously described methods [25].

Results

Cyclic-RGD increased the transduction efficiency in a sequence, conformation, and concentration-dependent manner (Fig. 1). For example, 1 day post transduction hMSCs treated with cyclic-RAD peptides had a transduction efficiency of 6.3% ± 1.0%, hMSCs treated with linear- RGD peptides had a transduction efficiency of 15.4% ± 10%, and hMSCs treated with cyclic-RGD peptides had a transduction efficiency of 76.6% ± 4% (Fig. 1A). Increasing the cyclic-RGD concentration increased the fraction of hMSCs expressing GFP (Fig. 1B). At 2 days post-transduction, hMSCs maintained in the growth medium had a transduction efficiency of 14.2% ± 4.7%, whereas hMSCs exposed to 86 μM cyclic-RGD peptides had a transduction efficiency of 78.4% ± 18.8%. Similar trends were observed when the transduction efficiency was measured using both imaging and flow cytometry-based techniques (Fig. 1C). The transduction efficiency of hMSCs treated with high concentrations of cyclic-RGD remained high over the course of the 7-day experiment. Alternatively, the transfection efficiency of untreated hMSCs increased over time, but did not achieve as high a transduction efficiency as hMSCs treated with cyclic-RGD

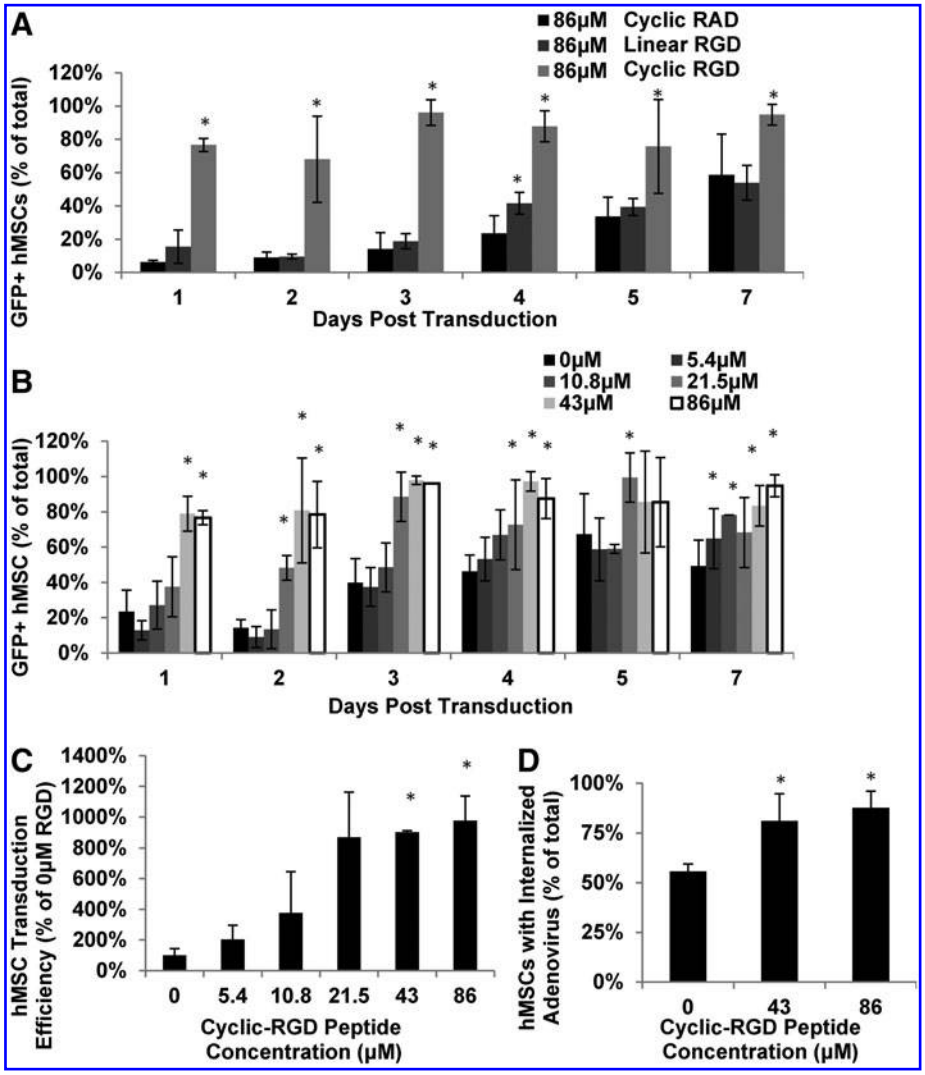

FIG. 1. Cyclic-RGD peptides influence on the adenoviral transduction of human mesenchymal stem cells (hMSCs). **(A)** Effect of amino acid sequence and conformation on the adenoviral transduction efficiency of hMSCs. * Denotes significant increase from 86 μM cyclic-RAD (*t*-test, $P < 0.05$). Error bars indicate standard deviation. **(B)** Effect of cyclic-RGD peptide concentration on the adenoviral transduction efficiency of hMSCs. * Denotes significant increase from 0 μM cyclic-RGD (*t*-test, $P < 0.05$). Error bars indicate standard deviation. **(C)** Normalized transduction efficiency of hMSCs treated with predetermined concentrations of cyclic-RGD peptides measured using a flow cytometer. * Denotes significant increase from 0 μM cyclic-RGD (*t*-test, $P < 0.05$). Error bars indicate standard deviation. **(D)** Effects of cyclic RGD peptides on the uptake of adenoviruses into hMSCs. * Denotes significant increase from 0 μM cyclic-RGD (*t*-test, $P < 0.05$). Error bars indicate standard deviation.

peptides. For example, after 7 days, untreated hMSCs had a transduction efficiency of 49.2%±14.7%, whereas hMSCs treated with 86 μM cyclic-RGD peptides had a transduction efficiency of 94.8%±6.3% (Fig. 1B). Also, the fraction of hMSCs that internalized adenoviruses increased from 55.7%±3.7% when they were maintained in the growth medium to 87.6%±8.5% when they were exposed to 86 μM cyclic-RGD (Fig. 1D).

After determining that the cyclic structure and the RGD peptide sequence were both necessary to increase hMSC transduction efficiency, we sought to determine if endocytosis contributed to the ability of cyclic-RGD peptides to enhance transduction. The role of endocytosis was studied by blocking the endocytotic uptake of reporter molecules with pharmacologic inhibitors. The pharmacological inhibitor concentrations used in this study significantly decreased the internalization of the small molecule fluorophore, Lucifer Yellow (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/scd), which suggested that these molecules and concentrations would be appropriate for the characterization of the effects of cyclic-RGD on endocytosis. One-day post-transduction 43 μM cyclic-RGD increased the transduction efficiency to 57.1%±6.6%. In contrast, when hMSCs were exposed to Dynasore and 43 μM cyclic-RGD, they had an adenoviral transduction efficiency of 2.1%±2.5% (Fig. 2A). Unlike previously observed trends (Fig. 1B), increasing the concentration of cyclic- RGD did not induce greater hMSC transduction efficiencies when endocytosis was blocked with Dynasore. Even 7-days post-treatment, hMSCs treated with Dynasore and 43 μM cyclic-RGD had a transduction efficiency of 0.8%±1% (Fig. 2A). The endocytosis inhibitor cytochalasin B also significantly decreased the ability of cyclic-RGD to enhance the transduction of hMSCs ($P<0.05$). Seven days post-transduction, hMSCs treated with cytochalasin B and 43 μM cyclic-RGD or 86 μM cyclic-RGD had transduction efficiencies of 2.8%±2.7% and 2.9%±4.0%, respectively. Alternatively, hMSCs treated with 43 μM cyclic-RGD alone had a transduction efficiency of 34%±17% (Fig. 2B).

After demonstrating that endocytosis contributed to the ability of cyclic-RGD peptides to enhance the Ad-GFP transduction of hMSCs, it was critical to test if these results would extend to therapeutically relevant proteins. Significantly, cyclic-RGD peptides enhanced the adenoviral-induced production of BMP2 (Fig. 3). hMSCs maintained in the growth medium treated with Ad-GFP or treated with cyclic-RGD peptides produced lower BMP2 concentrations compared to hMSCs transduced with Ad-BMP2 (Fig. 3A). In contrast, when hMSCs were treated with cyclic- RGD peptides and Ad-BMP2, they produced 413±192 ng/mL BMP2 in a medium collected from day 5–7, which was a significantly greater ($P<0.05$) BMP2 concentration than when they were treated with Ad-BMP2 alone.

The hMSC-produced BMP2 induced osteogenic gene expression in C2C12 cells (Fig. 3B, C). The conditioned medium from Ad-BMP2-transduced hMSCs significantly increased the fraction of C2C12 cells expressing alkaline phosphatase compared to C2C12 cells grown in the conditioned medium from untreated hMSCs ($P<0.05$). C2C12 cells exposed to a medium that was conditioned by hMSCs treated with Ad-BMP2 and cyclic-RGD had a significantly greater alkaline phosphatase activity than the medium from both untreated hMSCs and hMSCs transduced with Ad-BMP2 alone ($P<0.05$). In contrast, C2C12 cells treated with the conditioned medium from hMSCs exposed to Ad-BMP2 with cyclic-RAD had an increased alkaline phosphatase activity compared to the medium from untreated hMSCs, but did not have a significantly greater alkaline phosphatase activity

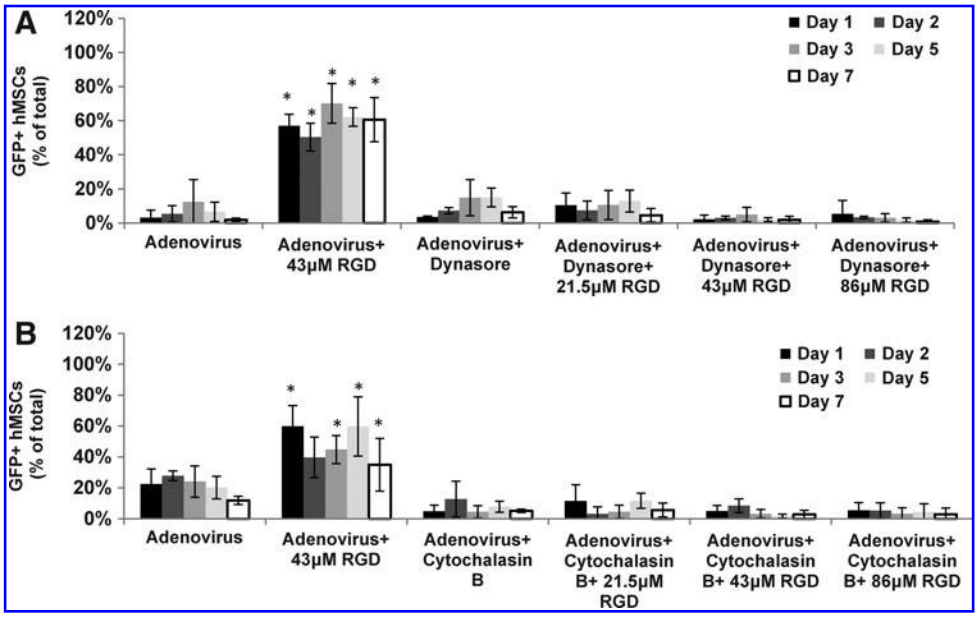

FIG. 2. Cyclic-RGD mediated increase in the adenoviral transduction efficiency of hMSCs using small molecule inhibitors of endocytosis. **(A)** Effect of endocytosis inhibitor, Dynasore, and cyclic-RGD peptides on the adenoviral transduction efficiency of hMSCs. * Denotes significant difference from treatment with adenovirus alone (*t*-test, $P<0.05$). Error bars indicate standard deviation. **(B)** Effect of cytochalasin B, another endocytosis inhibitor and cyclic-RGD peptides on the adenoviral transduction efficiency of hMSCs. * Denotes significant difference from treatment with adenovirus alone (*t*-test, $P<0.05$). Error bars indicate standard deviation.

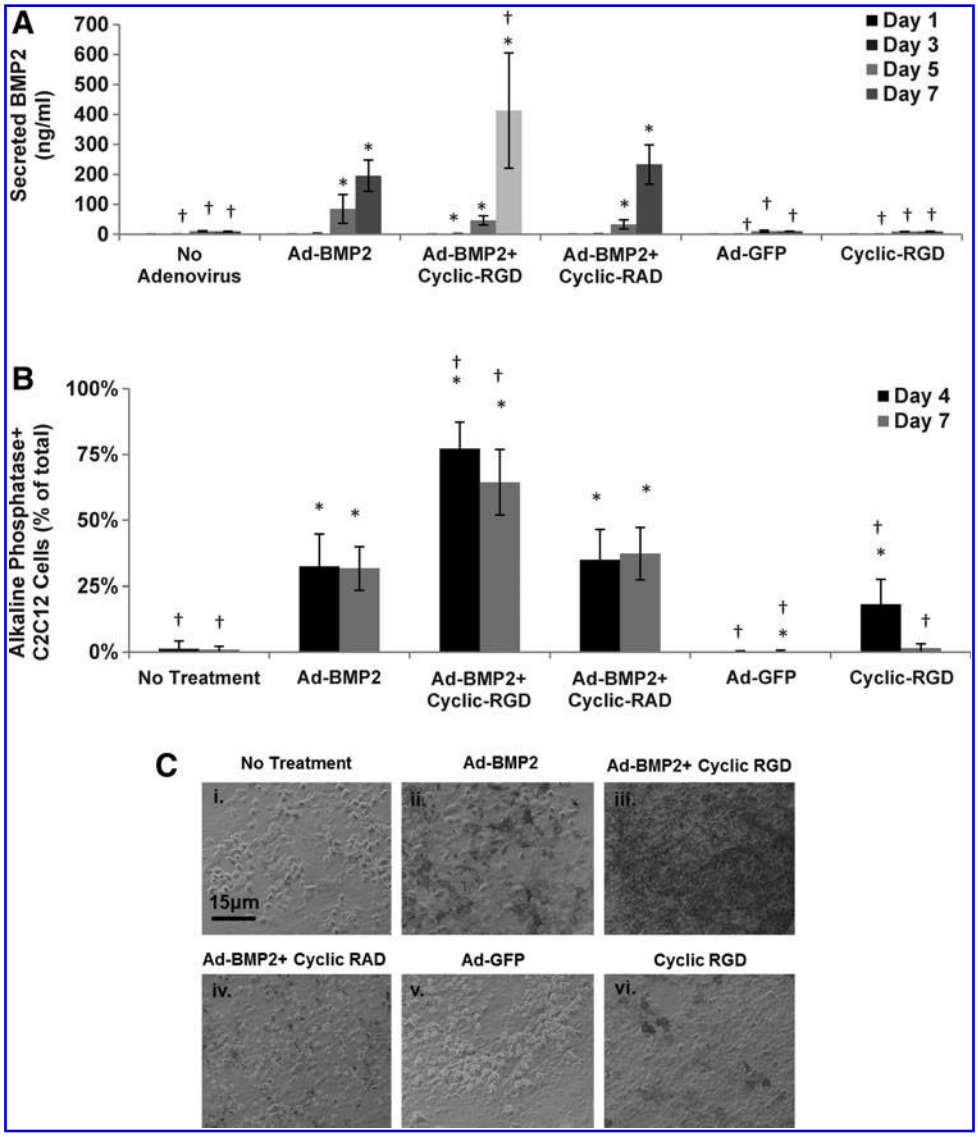

FIG. 3. Effect of cyclic-RGD peptides on the Ad-BMP2-induced expression of BMP2. **(A)** BMP2 concentration produced by hMSCs maintained in growth medium, treated with Ad-BMP2, Ad-BMP2 + cyclic-RGD, Ad-BMP2 + cyclic-RAD, Ad-GFP, or cyclic-RGD. **(B)** Percent of alkaline phosphatase expressing C2C12 cells treated with conditioned media from hMSCs exposed to growth media, Ad-BMP2, Ad-BMP2 + cyclic-RGD, Ad-BMP2 + cyclic-RAD, Ad-GFP, or cyclic-RGD. * Denotes significant difference from hMSCs maintained in growth media. Error bars indicate standard deviation. **(C)** Representative micrographs of C2C12 cells exposed to conditioned media from hMSCs treated with growth media, Ad-BMP2, Ad-BMP2 + cyclic-RGD, Ad-BMP2 + cyclic RAD, Ad-GFP, or cyclic-RGD. BMP2, bone morphogenetic protein-2. †, Denotes significant difference from hMSCs transduced with Ad-BMP2 (*t*-test, $P < 0.05$).

compared to conditioned media from hMSCs treated with Ad-BMP2 alone ($P < 0.05$). The medium collected from hMSCs transduced with Ad-GFP did not significantly increase the percentage of C2C12 cells expressing alkaline phosphatase. C2C12 cells exposed to the conditioned medium from hMSCs treated with cyclic-RGD had significantly increased alkaline phosphatase activity compared hMSCs maintained in growth media from days 1–4 ($P < 0.05$), but did not increase alkaline phosphatase activity from days 4–7. C2C12 cells exposed to the conditioned medium from hMSCs treated with cyclic-RGD induced significantly less alkaline phosphatase activity compared to media from hMSCs treated with Ad-BMP2 ($P < 0.05$) (Fig. 3B, C, i–vi). When C2C12 cells were grown in the C2C12 medium with increasing concentrations of cyclic-RGD peptides, no increase in the expression of alkaline phosphatase was observed (Supplementary Fig. S2, i–vi).

Discussion

Approaches to enhance the adenoviral transduction of hMSCs could be broadly used to enhance their drug delivery capabilities and therapeutic potential. hMSCs have internalized adenoviruses carrying genes to induce their production of therapeutic proteins [6]. However, hMSCs have been difficult to transduce with adenoviruses and required MOI's greater than 1,000 [21,26] and up to 3,000 [12]. Therefore, in this study we explored the effects of RGD-peptides on the internalization and transduction efficiency of therapeutic viruses. We characterized the effects of peptide sequence, conformation, and concentration on the adenoviral transduction efficiency of hMSCs and the production of biological drugs. Our results suggest that each of these parameters affects the adenoviral transduction efficiency of hMSCs and cyclic-RGD peptides may be broadly useful in increasing their therapeutic capabilities.

Cyclic-RGD peptides increased the internalization of model therapeutic viruses into hMSCs. Cyclic-RGD peptides have been covalently immobilized or genetically engineered into polymers [27], proteins [28], and therapeutic viruses to increase their internalization into cells [29]. However, these approaches could denature the biomolecules and have required reaction and purification schemes. Therefore, we sought to further characterize the effects of solution-phase

cyclic-RGD peptides on enhancing the transduction of hMSCs with therapeutic adenoviruses.

Peptides that had the RGD sequence in a cyclical structure increased the adenoviral transduction efficiency of hMSCs in a concentration-dependent manner. Previous studies have demonstrated that linear-RGD peptides inhibited the adenoviral transduction of hMSCs. However, these concentrations were 349% and 1977% greater than the highest concentrations of linear-RGD used in the experiments performed in this study [17]. Cyclic-RGD may have been more effective than linear-RGD in enhancing the transduction of hMSCs due to its enhanced stability or its increased affinity for $\alpha_v\beta_3$ integrins (Fig. 1A). Cyclic-RGD peptides have been measured to be 30 times more stable than linear-RGD peptides at pH 7 [30]. Furthermore, the cyclic-RGD peptide used in this study had a lower IC_{50} of 0.1–1.0 μM when compared to the linear-RGD peptide, which had an IC_{50} of 4–100 μM for the inhibition of cell adhesion to recombinant laminin fragments [31]. It will be critical to understand the stability and binding kinetics of these peptides in future studies, as both peptides and adenoviruses undergo rapid degradation in cell culture environments.

Cyclic-RGD peptides may have increased the internalization of adenoviruses through the dynamin-mediated endocytosis pathway (Fig. 2). Blocking dynamin inhibits phagocytosis, caveolar endocytosis, and clathrin-dependent endocytosis [32]. Understanding which internalization pathway is used by different biomacromolecules could have significant consequences, as different internalization pathways have resulted in different functional outcomes. For example, Dhaliwal and colleagues demonstrated that mouse MSCs cultured on fibronectin internalized plasmid DNA-polyethyleneimine particles primarily through endocytosis and these MSCs had significantly greater transgene expression than mouse MSC grown on collagen-I, which internalized particles primarily through macropinocytosis and caveolae-mediated endocytosis [33]. Understanding not only the internalization pathway, but also how the hMSCs' environment affects internalization will be important for drug delivery applications. Recent studies have characterized the effect of the microenvironment on the internalization of biomacromolecules into stem cells. For example, the effect of nanotopography [34] and encapsulation in biomaterials [35] has been explored on the internalization of adenoviruses. For example, hMSCs transduced with Ad-BMP2 in three-dimensional (3D) alginate hydrogels produced 7.41-fold more BMP2 than when they were transduced first in 2D culture, and then encapsulated in 3D hydrogels [35]. These approaches could be applied with the solution-phase cyclic-RGD-mediated approach delineated in this manuscript to further enhance the transduction efficiency of hMSCs.

Cyclic-RGD peptides increased the concentration of BMP2 produced by hMSCs after transduction with Ad-BMP2. Significantly, the concentration of BMP2 secreted by hMSCs in this study, 0.41 ± 0.19 μg/mL, was similar to or greater than the BMP2 concentration included in gelatin hydrogel disks to induce bone formation in preclinical animal studies (Fig. 3A) [36], which suggests that this technique could be extended to animal testing in future studies. Also, it is significant to note that the BMP2 produced by hMSCs in this study was able to induce alkaline phosphatase expression in C2C12 cells, which have been the canonical assay for BMP2 bioactivity (Fig. 3B, C) [23]. Protein overexpression could lead to misfolding and the production of proteins that do not illicit the desired therapeutic response [37]. A potential advantage of this approach is that cyclic-RGD and therapeutic adenoviruses could be included in the work flow of bedside stem cell purifying machines that are currently undergoing clinical trials, which could remove the time and expense of ex-vivo manipulation [38]. Therefore, this approach could be broadly applicable due to the ability of adenoviruses to carry genes for a wide variety of therapeutic proteins.

The conditioned medium from hMSCs treated with cyclic-RGD peptides alone increased alkaline phosphatase expression in C2C12 cells (Fig. 3C, i–vi), whereas C2C12 cells treated directly with cyclic-RGD peptides did not have an increased alkaline phosphatase activity (Supplementary Fig. S2). This finding suggested that the peptides may have induced hMSCs to secrete one or more pro-osteogenic factors. Several studies have demonstrated that cyclic-RGD peptides alone can induce a pro-osteogenic response, but when presented differently than in the culture medium used in this study. In one study, hMSCs cultured in alginate hydrogels with covalently immobilized linear RGD peptides were unable to induce osteocalcin expression, whereas covalently immobilized cyclic-RGD peptides induced osteocalcin expression and secretion [39]. In an in vivo sheep model, cyclic-RGD was as effective at inducing spine fusion as BMP2 when each drug was delivered from a mineralized collagen matrix [40]. Multiple secreted molecules have been implicated in directing stem cell osteogenic differentiation, including BMP family members [41], glucocorticoids [42], parathyroid hormone [43], and oxysterols [44]. Furthermore, recent studies have identified hundreds of proteins in hMSCs' secretome during medium-induced osteogenic differentiation [45,46]. Therefore, in future studies, it will be interesting to harness these techniques to measure the change in proteins secreted by hMSCs when they interact with cyclic-RGD peptides presented in different contexts.

Conclusions

In this manuscript, we characterized the use of cyclic-RGD peptides to enhance the transduction efficiency of adenoviruses. The enhanced uptake of therapeutic viruses was specific for peptides with the cyclic structure and RGD amino acid sequence. The increased transduction efficiency was dependent upon the peptide concentration in the culture medium. Blocking endocytosis with pharmacological agents inhibited the cyclic-RGD-mediated increase in adenoviral transduction efficiency. As a proof of therapeutic principle, cyclic-RGD peptides increased the adenoviral-induced production of an important therapeutic protein, BMP2. The enhanced BMP2 concentrations produced by hMSCs increased the alkaline phosphatase expression in C2C12 cells, which have been extensively used to measure BMP2 bioactivity. Taken together, this approach to enhancing the adenoviral transduction efficiency of hMSCs here could be used to increase the drug-delivering capability of hMSCs in future studies.

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Author Disclosure Statement

The authors declare that no competing interests exist.

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