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Fiber-modified adenovirus vectors mediate efficient gene transfer into undifferentiated and adipogenic-differentiated human mesenchymal stem cells

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Abstract

Human mesenchymal stem cells (hMSCs) are considered a source of cells for regenerative medicine, and cell and gene therapy. Efficient gene transfer into hMSCs is essential for basic investigations into cellular differentiation and developmental biology, and for therapeutic applications in gene-modified regenerative medicine. In the present study, we optimized the transduction of hMSCs by means of fiber-modified adenovirus (Ad) vectors. Among the various types of Ad vectors tested, the polylysine modification of the C-terminal of the fiber knob most markedly improved the efficiency of hMSC transduction. At 300 vector particles per cell of polylysine-modified Ad vectors, more than 95% of the hMSCs expressed transgene. In this condition, polylysine-modified Ad vectors mediated 460-fold more transgene activity than the conventional Ad vectors. Ad vectors containing the Ad type 35 fiber or an Arg-Gly-Asp (RGD) peptide in the fiber knob mediated 130 or 16 times, respectively, the transgene activity mediated by the conventional Ad vectors. We also examined the efficiency of transduction into adipogenic-differentiated hMSCs. In this latter case, only Ad vectors containing the Ad type 35 fiber showed efficient gene expression. These results showed that fiber-modified Ad vectors could become a potent tool for basic research into, and the therapeutic application of, hMSCs and adipogenic-differentiated hMSCs.

Keywords: Adenovirus vector; Mesenchymal stem cells; Adipocytes; Gene therapy; Regenerative medicine

Bone marrow-derived mesenchymal stem cells (MSCs) have high proliferative capacity [1] and can differentiate into adipocytes, osteoblasts, and chondrocytes [2]. They can also differentiate into other types of cells such as nerve cells [3,4] and hepatocytes [5]. MSCs are considered vehicles for cell and gene therapy. As vehicles for cell therapy, MSCs are directly injected into the mesenchymal tissues, because these cells are progenitors of mesenchymal tissues. As vehicles for gene therapy, genetically modified MSCs are delivered systemically or injected directly to tissues of interest to express therapeutic proteins in the desired tissues. To generate genetically modified MSCs, it is essential to use a vector that efficiently mediates gene transfer into MSCs. An efficient gene transfer vector is also essential for basic research into MSCs, such as analyses of cellular differentiation and developmental biology.

Recombinant adenovirus (Ad) vectors continue to be the preferred vectors for gene therapy and the study of gene function. They are relatively easy to construct, can be produced at high titers, and have high transduction efficiencies. The efficiency of Ad vector-mediated transduction into human MSCs (hMSCs), however, is

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quite low due to the scarcity of the primary receptor, called the coxsackievirus and adenovirus receptor (CAR) [6–9]. Therefore, hMSCs usually have been transduced with high titers (more than 1000 infectious units/cell) of Ad vectors [6,8,9]. Fiber-modified Ad vectors overcome this obstacle. We and other groups have developed several types of fiber-modified Ad vectors. One is constructed by the addition of foreign peptides to the HI loop or C-terminal of the fiber knob of an Ad vector [10–14]. Enhanced gene transfer has been reported, based on the use of mutant fiber proteins containing either an Arg-Gly-Asp (RGD) peptide [10–15] or a stretch of lysine residues (K7 (KKKKKKK) peptide) [10,14,15], which, respectively, target αv integrins or heparan sulfates on the cellular surface. Another type of fiber-modified Ad vector is made by removing fibers from one Ad serotype (Ad type 5) and replacing them with fibers derived from another—specifically, fibers that bind to receptor molecules other than CAR [16–20]. That is, fiber proteins derived from Ad belonging to the subgroup B, such as Ad type 3, 11, and 35, replace the Ad type 5 fiber. These fiber-modified Ad vectors infect cells via CD46, CD80, or CD86, which are recently identified cellular receptors of Ad belonging to subgroup B [21–25].

In the present study, we optimized the transduction to hMSCs by Ad vectors containing an RGD peptide in the HI loop of the fiber knob, Ad vectors containing a polylysine peptide in the C-terminal of the fiber knob, and Ad vectors containing a fiber protein derived from the Ad type 5 fiber tail, and the Ad type 35 fiber knob and shaft. The results showed that polylysine modification of the fiber knob greatly improved the efficiency of Ad vector-mediated transduction into hMSCs. We also report the efficient gene transfer into adipogenic-differentiated hMSCs by the Ad vectors containing Ad type 35 fiber.

Materials and methods

Ad vectors. Ad vectors expressing an *Escherichia coli* β-galactosidase (LacZ) were constructed by an improved in vitro ligation method [26,27]. The shuttle plasmid pHMCA-LacZ1 contains a CA promoter (a β-actin promoter/CMV enhancer with a β-actin intron) [this promoter/enhancer was kindly provided by Dr. J. Miyazaki (Osaka University, Osaka, Japan)] [28], the LacZ gene derived from pCMVβ (Clontech, Palo Alto, CA, USA), and a bovine growth hormone polyadenylation signal, all of which are flanked by I-CeuI and PI-SceI sites. I-CeuI/PI-SceI-digested pHMCA-LacZ1 was ligated with I-CeuI/PI-SceI-digested pAdHM4 [26], resulting in pAdHM4-CALacZ1. pAdHM15-RGD-CALacZ1, pAdHM41-K7-CALacZ1, and pAdHM34-CALacZ1 were constructed by the ligation of I-CeuI/PI-SceI-digested pHMCA-LacZ1 with I-CeuI/PI-SceI-digested pAdHM15-RGD [13], pAdHM41-K7 [14], and pAdHM34 [20], respectively. Viruses (Ad-CALacZ, AdRGD-CALacZ, AdK7-CALacZ, and AdF35-CALacZ) were generated with the transfection of PacI-digested pAdHM4-CALacZ1, pAdHM15-RGD-CALacZ1, pAdHM41-K7-CALacZ1, and pAdHM34-CALacZ1, respectively, into 293 cells per virus with SuperFect (Qiagen, Valencia, CA) according to the manufacturer's instructions. Each virus was purified by $CsCl_2$ step gradient ultra-centrifugation followed by $CsCl_2$ linear gradient ultra-centrifugation. The virus particles and biological titer were determined spectrophotometrically by the method of Maizel et al. [29] and by using an Adeno-X Rapid Titer Kit (Clontech, Palo Alto, CA), respectively. The ratio of biological-to-particle titer was 1:22 for Ad-CALacZ, 1:26 for AdRGD-CALacZ, 1:32 for AdK7-CALacZ, and 1:21 for AdF35-CALacZ. The Ad vectors used in the present study were summarized in Table 1.

Cells. Bone marrow-derived hMSCs [purchased from Cambrex Bio Science Walkersville (Walkersville, MD)] were cultured with mesenchymal stem cell basal medium (MSCGM) (Cambrex Bio Science Walkersville) according to the manufacturer's instructions. hMSCs were used for experiments during passages two to four.

Adipocyte differentiation. The adipogenic-differentiated hMSCs were induced according to the manufacturer's instructions (Cambrex Bio Science Walkersville). In brief, hMSCs were seeded at a density of 2.1×10^4 cells/cm^2 and cultured with MSCGM for 10 days. The cells were then cultured with supplemented adipogenesis induction medium (Cambrex Bio Science Walkersville) for 3 days followed by 3 days of culture in supplemented adipogenesis maintenance medium (Cambrex Bio Science Walkersville). After three cycles of induction and maintenance, the cells were cultured with supplemented adipogenesis maintenance medium for 7 days. The differentiation of hMSCs to adipocytes was monitored by measuring intracellular lipid accumulation using Oil red O staining. In brief, the cells were fixed for 2 h with 10% formaldehyde in isotonic phosphate buffer and then washed with distilled water. The cells were then stained with complete immersion in a working solution (0.3%) of Oil red O for 4 h. Excess dye was removed by exhaustive washing with water.

Adenovirus-mediated gene transduction in vitro. hMSCs (1.1×10^4 cells) were seeded into a 24-well dish and the next day the cells were treated with each Ad vector for 1.5 h. Then, the medium containing the vectors was removed and fresh medium (MSCGM) was added to the cells. At the indicated amount of time, LacZ activity in the cells was measured by both X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) staining or the luminescence assay using a luminescent β-galactosidase genetic reporter system II (Clontech).

Flow cytometry. To detect human CAR, the cells (5×10^5 cells) were labeled with mouse monoclonal antibody RmcB (anti-human CAR) (Upstate Biotechnology, Lake Placid, NY) and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG secondary antibody (Pharmingen, San Diego, CA). To detect human CD46, the cells were labeled with FITC-conjugated anti-human CD46 antibody (Pharmingen). Flow cytometric analysis was performed by a FACSCalibur flow cytometer using CellQuest software (Becton–Dickinson, Tokyo, Japan).

Results and discussion

To develop suitable Ad vectors for hMSCs, various types of fiber-modified Ad vectors expressing LacZ,

Table 1
Adenovirus vectors used in the present study

Name	Fiber type	Promoter
Ad-CALacZ	Type 5 fiber	CA promoter
AdRGD-CALacZ	RGD peptide in the HI loop of the fiber knob	CA promoter
AdK7-CALacZ	Polylysine peptide in the C-terminal of the fiber knob	CA promoter
AdF35-CALacZ	Chimeric type 5 fiber tail and type 35 fiber knob and shaft	CA promoter

CA promoter: β-actin promoter/CMV enhancer with β-actin intron.