Viral Vectors for Stable Transduction of Human Mesenchymal Stem Cells: Systems Based on Adeno-Associated Viruses and Lentiviruses

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We compared the efficiency of transduction with lentivectors based on HIV-1 and adeno-associated viruses serotype 2 and stability of transgene expression in human mesenchymal bone marrow stem cells.

Key Words: *mesenchymal stem cells; lentivirus vectors; transduction*

The prospects of clinical use of mesenchymal stem cells (MSC) from human bone marrow (BM) modified by therapeutically useful transgenes dictate the need in evaluation of the most effective system for transgene delivery. The advantage of vectors based on recombinant lentiviruses and adeno-associated viruses is stable transduction and long-term expression of the transgene in primary cell cultures including MSC.

The aim of the present study was to compare the efficiency of transduction with lentivectors based on HIV-1 and adeno-associated viruses serotype 2 and stability of transgene expression in MSC from human BM.

MATERIALS AND METHODS

Vector plasmids used for virus assembly were generated in *Escherichia coli* strain DH5a under standard conditions (medium LB containing 10 g/liter tryptone, 10 g/liter NaCl, and 5 g/liter yeast extract, pH 7.2, 100 mg/liter ampicillin, in dark, at 37°C). Preparative isolation and purification of plasmids for cell transfection were performed using Endo-Free Plasmid Maxi kits (Qiagen) according to manufacturer's instructions.

For isolation of MSC, BM aspirate was separated by centrifugation in Ficoll gradient, the mononuclear fraction was collected, washed twice with PBS, and seeded in MEM medium (α-modification) supplemented with 10% fetal calf serum (FBS; HyClone) [1]. MSC selected by adhesion to plastic were immunotyped using antibodies to CD90, CD105, CD45, and CD34. The cells were cultured in a CO_2 incubator (5% CO_2) at 37°C. The growth medium was replaced after 2 days. HEK 293T cells were cultured under similar conditions in DMEM with 10% FBS (HyClone).

Transfection of HEK 293T cells was carried out in 6-well plates using ExGen 500 reagent (Fermentas) or by the calcium phosphate method. In transduction experiments, supernatant of the producent strain purified from the cells and debris by filtration was used as the source of recombinant viruses. Supernatant of a strain co-transfected with a transfer vector and one of the two necessary helper plasmids served as the control. GFP fluorescence was detected on a Carl Zeiss Axio Imager M1 microscope. The percent of transfected and transduced cells was determined on a FACScan cytometer (BD).

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RESULTS

Transfection of HEK 293T cells with components of the three-plasmid systems yielded recombinant adeno-associated viruses (AAV) and lentiviruses (HIV); their titers were determined by transduction of HEK 293T cells in serial dilutions. The production of AAV2 was provided by transfection of HEK 293T cells by pAAV-IRES-hrGFP transfer vector in combination with plasmids pAAV-RC (responsible for expression of Rep and Cap, *in trans* proteins of adeno-associated virus) and pHelper (responsible for expression of helper adenoviral proteins E2A, E4, and V2 RNA) Recombinant lentiviruses HIV-1 were obyained by co-transfection of HEK293T cells by transfer vector pHR-SINcPPT-SIEW in combination with plasmids pCMVdelta-R8.91 (encodes GAG/POL, Tat, and Rev proteins) and pMD2.G (encodes VSV-G protein responsible for pseudotyping of viral envelope). Transfer vectors of lentiviral and AAV systems carry reporter GFP gene, an effective marker of delivery and expression of the transgene construct.

The obtained viral stocks were used for transduction of MSC from human BM at MI=10 (multiplicity of infection; 10 viral particles per cell). Both the lentivirus-based and AAV systems demonstrated the possibility of delivery of the specified transfer vectors to MSC (Fig. 1), stable expression of the reporter gene GFP, and multiple passaging. However, the efficiency of transduction with lentiviral vectors considerably (by 2 orders of magnitude) surpassed that for AAV at the same concentration of viral particles (MI=10) and was 94.0±2.8% *vs*. 2.86±1.60%. This can be explained by low concentration of heparin sulfate proteoglycan, AAV receptors, on the surface of MSC, peculiarities of the synthesis of the second DNA strand of viral genome, *etc*. [2-4,12].

The possibility of effective use of AAV-based vectors for transduction of MSC from human BM was demonstrated in previous studies [11]. However, in these experiments expression of GFP in transduced cells decreased practically to zero on day 8 after transduction. Our findings demonstrated stable expression of the transgene for at least 1 month. Effective transduction of MSC (up to 65% [11]) can be attained by increasing MI to 10,000, which can be associated with increased production of AAV and subsequent concentration of viral particles by centrifugation in $CsCl_2$ gradient. At the same time, transduction of human MSC with self-complimentary AAV with an efficiency of 66% at MI=1000 was shown, which resulted in stable expression *in vivo* for 3 months [7]. Flow cytometry using a fluorescence-activated cell sorter also enriches the population with GFP-expressing transduced cells to 95% and more [8].

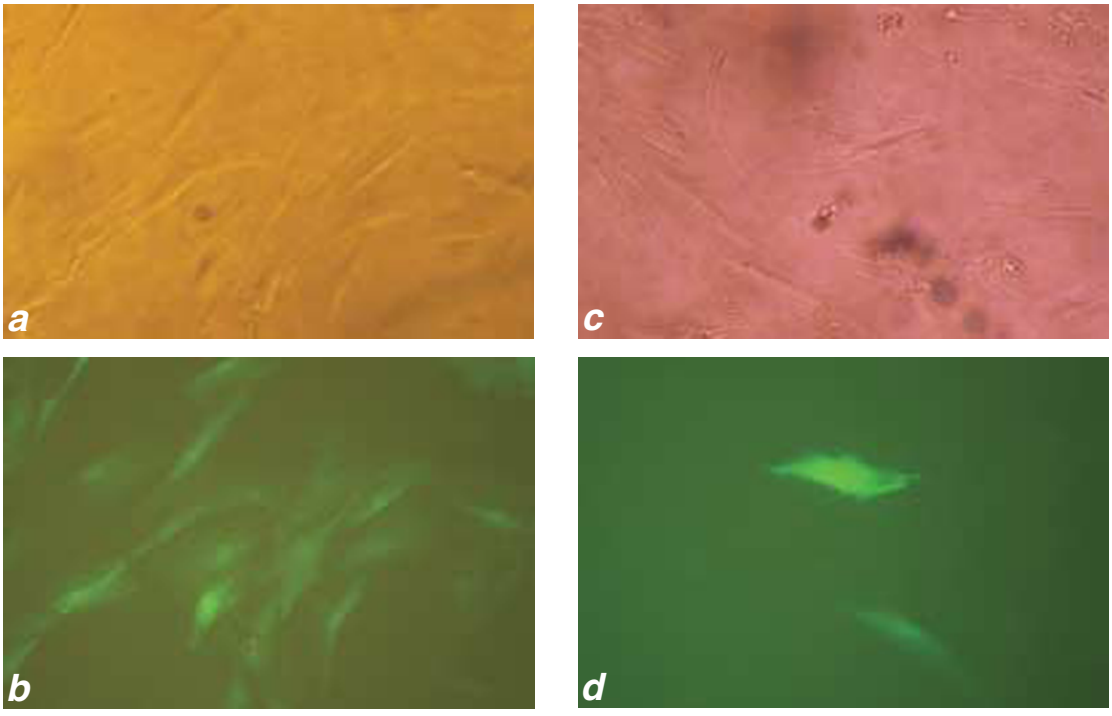

Fig. 1. Transduction of MSC from human BM with vectors based on lentivirus HIV-1 (*a, b*) and AAV2 (*c, d*). *a, c*: visible light; *b, d*: GFP fluorescence.

At the same time, the use of HIV-based lentivirus vectors provided effective and convenient delivery of the transgene to MSC and its long-term and stable expression, which was confirmed by a number of studies [6,13,14]. Potential drawbacks of lentiviruses is accidental localization of transgene insertion, which can lead to genome destabilization, and hypothetical possibility of reversion of the virus to wild type, whereas AAV causes no diseases and introduces the transgene into the studied locus AAVS1 in chromosome 19.

Systems based on oncoretroviruses, adenoviruses, lentiviruses, α-viruses, herpesviruses, baculoviruses, *etc*. are actively used for transduction of cell cultures [5,6,9,10,13,14]. The advantage of the discussed lentivirus- and AAV-based vectors are their wide spectrum of activity, low toxicity, and, most important, the capacity to integrate the transgene into the cell genome providing its long-term expression. Further adaptation of methods of viral transduction of MSC for stable expression of the transgene is an important stage in the development of strategies of gene and cell therapy with the use of MSC.

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