Cytotherapy, 2014; 16: 612–618

International Society for Cellular Therapy
ISCT®

Evaluation of optimal concentration and exposure duration of valproic acid alone or in combination with ViraDuctin to augment adenovirus transduction in human adipose stem cells

BABAK NEGAHDARI[1,2], MOHAMMAD REZA KHORRAMIZADEH[3], MOHAMMAD HOSEIN MODARRESI[4], CHRISTINE HARTOONIAN[5], MOHAMMAD ALI SHOKRGHOZAR[6] & KAYHAN AZADMANESH[1]

[1]Department of Virology, Pasteur Institute of Iran, Tehran, Iran, [2]School of Advanced Technologies in Medicine, Department of Medical Biotechnology, Tehran University of Medical Sciences, Tehran, Iran, [3]Endocrinology and Metabolic Research Institute of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran, [4]Medical Genetics Department, Tehran University of Medical Sciences, Tehran, Iran, [5]Department of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Tehran University of Medical Sciences, Tehran, Iran, and [6]National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran

Abstract

Background aims. Recombinant adenoviruses have tremendous potential in both gene therapy research and therapeutic applications. Mesenchymal stromal cells have a set of several properties that make them ideally suited for both regenerative medicine and gene and drug delivery. A limitation of adenoviral-mediated gene transfer is indeed the poor transduction rate of cells with low or no levels of the specific adenoviral cell surface receptor coxsackie virus and adenovirus receptor (CAR), such as human mesenchymal stromal cells. In the present work, we tried to increase the adenovirus transduction level and mediated gene delivery of human adipose stem cells with the use of valproic acid (VPA) and determined the proper concentration and duration of treatment alone or in combination with ViraDuctin adenovirus transduction reagent. *Methods.* Green fluorescent protein–expressing recombinant adenovirus was propagated. The effects of various doses and exposure periods of VPA on CAR expression in human adipose stem cells were speculated by quantitative real-time polymerase chain reaction and adenoviral transduction rate by flow cytometry in different doses and time intervals of VPA and in combination with ViraDuctin transduction reagent. *Results.* CAR messenger RNA upregulation through VPA was observed in human adipose stem cells; it was a dependent factor of dose and exposure time. Consequently, adenoviral transduction level of human adipose stem cells treated with VPA was increased, and co-administration of VPA and ViraDuctin further enhanced the transduction rate. *Conclusions.* These results confirm that addition of VPA to hASCs alone or in combination with ViraDuctin has enhancing effects on adenoviral transduction rate, which can be auspicious in adenoviral-mediated gene therapy.

Key Words: *adenoviral vector, gene therapy, human adipose stem cell, valproic acid*

Introduction

Multipotent mesenchymal stromal cells (MSCs) are promising candidates in the field of regenerative medicine (1). The limiting step of the use of MSCs as a gene delivery vehicle is an efficient delivery method. Although the use of chemical methods to generate genetically modified MSCs has been reported, the most important concern is their very low efficiency (2). Viral-based gene delivery methods have shown promising results to address this issue (3). Reports indicate that in 28% of 600 gene therapy studies, adenovirus vectors have been used to deliver therapeutic or marker genes (4). For high-level and transient expression of a transgene, adenovirus vectors have become gene-transfer vehicles of choice. Binding/entry of Adenovirus type 5 to susceptible cells is mediated by two cell-surface receptors: coxsackie virus–adenovirus receptor (CAR) and cellular avß integrins. On many cell types, including most stem cells, both adenovirus receptors are absent or expressed at low levels, leading to poor transduction efficiency by adenovirus (4,5). It has been previously

Correspondence: **Kayhan Azadmanesh**, MD, PhD, Virology Department, Pasteur Institute of Iran, PO Box 1316942551, 12th Farvardin Street, Pasteur Avenue, Tehran, Iran. E-mail: azadmanesh@pasteur.ac.ir

(Received 23 December 2012; accepted 14 August 2013)

http://dx.doi.org/10.1016/j.jcyt.2013.08.015

shown that enhanced expression of CAR on cell lines increased the feasibility of gene transfer by adenovirus recombinants *in vitro* (6–9).

Two beneficial approaches have been taken toward addressing low transduction rates by adenovirus. Augmented CAR expression by use of valproic acid (VPA) and other histone deacetylase (HDAC) inhibitors has been reported previously in both *in vitro* and *in vivo* studies (5,9,10). The second approach is the use of ViraDuctin adenovirus transduction reagent (Cell Bio Labs Inc, San Diego, CA, USA) for transduction of cells with low or absent levels of CAR expression. ViraDuctin increases adenovirus transduction rates by a CAR-independent mechanism.

In the present study, the effect of VPA on the kinetics of CAR expression in human adipose stem cells (hASCs) was evaluated in different doses and exposure durations, and the consequences of CAR expression level on adenovirus infectivity rate were assessed. It was also shown that simultaneous application of ViraDuctin transduction reagent and VPA enhanced adenovirus transduction rate.

Methods

Production of green fluorescent protein–expressing adenoviruses

The AdenoVator vector system (Q.BIOgene, Carlsbad, CA, USA) was used to produce green fluorescent protein (GFP)-expressing adenovirus particles according to the manufacturer's instructions. Briefly, pAdenoVator-CMV5-IRES-GFP transfer vector was linearized with *Pme* I and co-transformed with pAdenoVator ΔE1/E3 adenovirus serotype 5 (Ad5) into BJ5183 *Escherichia coli* by means of electroporation. Recombinants resulting from homologous recombination and containing the GFP expression cassette were selected with kanamycin and screened by means of *BstX* I restriction enzyme analysis. The recombinant vector was cleaved by *Pac* I to expose its inverted terminal repeats. PolyFect transfection reagent (Qiagen, Hilden, Germany, 301105) was used to transfect linearized recombinant vector into human embryonic kidney (HEK) 293 cells (NCBI, Tehran, Iran, C497).Briefly, on the day before transfection, 1.2×10^5 HEK 293 cells per well were plated in 24-well cell culture plates (SPL Life Sciences Co, Ltd, Gyeonggi-do, Korea) in Dulbecco's modified Eagle's medium (DMEM) (PAA, Pasching, Austria) supplemented with 10% fetal bovine serum (FBS) (PAA), 100 IU/mL penicillin, 100 mg/mL streptomycin (PAA) and 4 mmol/L L-glutamine (PAA). Transfected cells were monitored for GFP expression by inverted fluorescent microscopy and harvested 17 days after transfection. After three cycles of freezing in an ethanol-dry ice bath and rapid thawing at 37°C, cell suspensions were centrifuged and supernatant containing recombinant viruses (summarized here as Ad5GFP) were supplemented with 10% glycerol and stored at −80°C.

Adenovirus amplification and titration

HEK 293 adenovirus packaging cells were cultured in DMEM supplemented with 5% FBS to reach 90% confluence. After aspiration of medium, adenoviral stock was added to cells and incubated for 2 h at 37°C. Fresh DMEM supplemented with 2% FBS was added, and cells were incubated until almost all cells lifted off the bottom of plates as representative of the cytopathic effect. For virus amplification, HEK 293 cells were re-infected four to five times sequentially by the infected cell lysates as described above. The final cell lysates were centrifuged briefly, and supernatant containing increased viral titer were supplemented with 10% glycerol and stored at −80°C. To calculate the viral titer, flow cytometry analysis was performed by use of the Cyflow apparatus (Partec, GmbH, Munster, Germany) and FloMax software (Quantum Analysis GmbH, version 2.70; Münster, Sentrup, Germany) as described elsewhere, with some modifications (11). Briefly, five serial 10-fold dilutions of virus stock were prepared in DMEM supplemented with 2% FBS. At a low multiplicity of infection, virus titer was represented as green fluorescent units (gfu)/mL and calculated as: titer (gfu/mL) = (% of GFP-expressing cells × cell number at infection time × dilution factor)/(100 × volume of diluted virus stock).

ASC isolation and characterization

Human ASCs were isolated from human adipose tissue as a waste of surgical procedure from the consenting patient. Adipose tissue was chopped into small pieces and digested with 0.02 mg/mL collagenase type I (Sigma, St Louis, MO, USA) for 1 h in an incubator (37°C, 5% CO_2). The suspension was centrifuged at 1000 rpm for 5 min, and the cell pellet at the bottom separated. The cell pellet was transferred to the DMEM supplemented with 10% FBS, 100 IU/mL penicillin and 100 mg/mL streptomycin in a humidified incubator (37°C, 5% CO_2). After 24 h, non-adhered cells were removed and fresh culture medium was replaced. To characterize stem cells, the so-called processed lipoaspirate (PLA)-adhered cells in early passages were evaluated for positive localization of cluster of differentiation (CD)73 and CD90 surface antigens as positive markers of hASCs and lack of hematopoietic lineage markers CD45, CD31 and CD11b by flow cytometry analysis (12). CD11b and CD31