Pharmacological Research 127 (2018) 49–57

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Optimized lentiviral transduction of human amniotic mesenchymal stromal cells

Federica Pisano[a,b,c], Manuela Mura[a,b], Maria Chiara Ciuffreda[a,b], Federica Calabrò[a,b], Nicola Lanzo[c], Massimiliano Gnecchi[a,b,c,d,*]

[a] Department of Internal Medicine and Infectious Diseases-Coronary Care Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[b] Laboratory of Experimental Cardiology for Cell and Molecular Therapy, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[c] Department of Molecular Medicine, Unit of Cardiology, University of Pavia, Italy
[d] Department of Medicine, University of Cape Town, South Africa

ARTICLE INFO

Article history:
Received 21 June 2017
Received in revised form 3 November 2017
Accepted 13 November 2017
Available online 14 November 2017

Keywords:
Lentivirus
Mesenchymal stromal cells
Gene therapy
Stem cell therapy
Heart disease

ABSTRACT

Mesenchymal stromal cells are excellent candidates for regenerative medicine since they are multipotent, easy to isolate, can be expanded to obtain clinically relevant numbers and are immunoprivileged. Stable genetic modification with viral vectors can improve mesenchymal stromal cell function and enhance their therapeutic potential. However, standard viral vectors achieve sub-optimal transduction efficiency with a single infection. On the other hand, multiple transduction cycles or antibiotic-based selection methods may alter the stem cell phenotype. We hypothesized that the use of lentiviral vectors containing specific regulatory sequences may result in improved transduction efficiency. Thus, we compared two types of third generation lentiviral vectors, one of which, the pLenti7.3 vector, contains the optimized sequences for Polypurine Tract and Woodchuck Post-transcriptional Regulatory Element. We demonstrated that with the pLenti7.3 it is possible to efficiently transduce human mesenchymal stromal cells with a single transduction cycle. Additionally, we successfully showed that by using the pLenti7.3 vector it is possible to efficiently over-express different growth factors, particularly relevant for cardiac protection and differentiation, in human mesenchymal stromal cells.

1. Introduction

Mesenchymal stromal cells (MSC) are immunomodulatory, multipotent and fast proliferating cells that have been tested as regenerative therapy [1,2]. The most active fields of MSC therapy include skin, bone, cartilage and cardiomyocyte (CMC) regeneration [3–6]. For instance, it has been shown that the injection of bone marrow derived MSC (BM-MSC) following acute myocardial infarction (AMI) results in significant reduction of the damaged regions and improvement in heart function [7–13]. However, BM-MSC do not lead to high levels of cardiac regeneration because of their low differentiation efficiency into CMC [14,15]. On the contrary, it has been proven that MSC mediate several beneficial paracrine mechanisms leading to heart repair after ischemic events [16]. Unfortunately, these paracrine properties may be impaired by donor age [17]. We have recently demonstrated that MSC of fetal origin isolated from the placental amniotic membrane (A-MSC) exert powerful paracrine effects but their cardio-regenerative potential, as in the case of adult BM-MSC, is limited [18,19]. For all these reasons, tools to increase MSC differentiation and/or paracrine effects are needed.

It is known that by over-expressing growth factors (GF) involved in cardiac differentiation, the potential of MSC to differentiate into CMC as well as their paracrine properties can increase [20–31]. The best method to permanently introduce genetic information into MSC is through viral transduction [32–37]. However, the infection efficiency is usually low and may be insufficient for differentiation purposes [38–40]. Viral vectors encoding for antibiotic resistance have been also tested but the selection of transduced cells with antibiotics may alter the stromal cell phenotype, as well as the growth rate and the viability of transformed cells [41–43]. Consequently, the development of highly efficient and less cytotoxic viral gene transduction systems is crucial for advancement of MSC-based regenerative medicine. In our study, we compared two types of third generation lentiviral vectors in order to identify the best method to transduce MSC without altering their phenotype, growth rate or viability.

* Corresponding author at: c/o Fondazione IRCCS Policlinico San Matteo, Viale Golgi 19, 27100 Pavia, Italy.
E-mail address: m.gnecchi@unipv.it (M. Gnecchi).

https://doi.org/10.1016/j.phrs.2017.11.014
1043-6618/

2. Material and methods

An expanded Methods section is available in the Supplementary material.

2.1. Generation of lentiviral plasmids

We generated two different plasmids encoding the insulin-like growth factor 1 (IGF1), that were used to identify the most efficient lentiviral vector for A-MSC infection. Then, we constructed other three plasmids, each one encoding a different GF, to confirm that the lentiviral vector identified in the first part of the study as the best vector was truly efficient and reliable for A-MSC transduction. The three GF selected were the bone morphogenic protein 2 (BMP2), the transforming growth factor β1 (TGFβ1) and the fibroblast growth factor 2 (FGF2). We followed the same methodology for the construction of all vectors. Specifically, the cDNA of each GF was obtained with a high fidelity DNA polymerase and the PCR products were adenosine-tailed. Sequences were cloned into the linearized pCR8/GW/TOPO vector with thymidine tails (at 5′ and 3′) by adding a Salt Solution (Life Technologies, Carlsbad, California, US) diluted 1:4 with DEPC water and incubating at room temperature for 30 min. The reaction mix was incubated on ice and transformed in competent One Shot Mach1-T1 *E. coli* cells using the protocol described in the "Bacterial competent cell transformation and culture" supplemental paragraph. Because the cloning protocol was non-directional, it was necessary to control the orientation of the cloned cDNA in the plasmid. The GW1 forward primer was used (GTTGCAACAAATTGATGAGCAATGC) (Life Technologies, Carlsbad, California, US) for all samples. It annealed to a pCR8/GW/TOPO sequence located less than 55 nucleotides from the 5′ PCR product insertion site. Reverse primers were designed to find homologous sequence on each GF cDNA: IGF1 reverse primer (CTACATCCTGTAGTTCTTGTTTCC), BMP2 reverse primer (GTCGCCCAGGGCCGGTGGTA), TGFβ1 reverse primer (TCAGCTGCACTTGCAGGAGCGC) and FGF2 reverse primer (TCAGCTCTTAGCAGACATTGGAAG). Once the correct clones were identified by PCR, they were used for the recombination reaction based on the Gateway Technology (Life Technologies, Carlsbad, California, US). The recombination protocol was performed following the manufacturer's instructions. Briefly, pLenti and pCR8/GW/TOPO-GF were mixed at a 1:1 ratio by adding LR Clonase enzyme (Life Technologies, Carlsbad, California, US) and were incubated at room temperature for 60 min. Finally, the mixture was transformed in One Shot Stbl3 *E. Coli* cells as described in the "Bacterial competent cell transformation and culture" in the supplementary methods. From bacterial cultures, pLenti7.3 plasmids were isolated and then controlled and used for our purposes. For additional information and protocol regarding the construction of plasmid vectors, please see the "Supplementary methods".

2.2. Lentivirus production and titration

At day 0, 293FT cells were plated into 175 cm^2 flasks at the density of 2×10^5 cells/cm^2. At day 2, the cells were washed twice with PBS 1X and cultured with medium without antibiotics. At day 3, cells were co-transfected over-night with Lipofectamine 2000 (Life Technologies, Carlsbad, California, US), 15 μg lentiviral expression plasmid and 30 μg Virapower® mix and incubated at 37 °C, 5% CO_2 overnight. At day 4, the transfection medium was replaced with fresh medium supplemented with 8% sodium butyrate (Sigma Aldrich, St. Louis, Missouri US) to enhance lentiviral transgene expression. Forty-eight and 72 h after co-transfection, viral particles were collected, purified from cellular debris and concentrated with 5X PEG-it virus precipitation solution (EuroClone Group, Milan, Italy). Titration was performed with p24 ELISA kit following the manufacturer's instructions (EuroClone Group, Milan, Italy).

Table 1
Characteristics of GF sequences cloned into pLenti.

Growth factor	Interspersed repeats	Blast
IGF-I	None	Amp^+ gene **ΔU3/3′ LTR**
BMP2	None	ccdB gene polyA-bla promoter
TGFβ1	None	GFP gene (x2) ccdB gene SV40-GFP
FGF2	None	pUC ori $Chloramphenicol^+$ GFP gene **WPRE**

2.3. Cell transduction

A preliminary experiment was performed with the virus expressing the IGF1 gene to identify the best multiplicity of infection (MOI) for each cell type. We used the following protocol. Both the IGF1-pLenti6 and IGF1-pLenti7.3 were diluted 10^{-3}, 10^{-4} and 10^{-5} times. At day 0, cells were plated and incubated in complete culture medium over-night at 37 °C, 5% CO_2. At day 1, each virus dilution was mixed with 8 μg/mL of hexadimethrine bromide (Polybrene®, Sigma Aldrich, St. Louis, Missouri US) and with culture medium and then used to feed 10^5 cells. At day 2, fresh medium was replaced and 48 h after transduction, green fluorescent protein (GFP) expression was analyzed with an inverted Zeiss Axio Observer Z1 microscope, equipped with ApoTome system (Carl-Zeiss, Oberkochen, Germany). For information and protocol about the isolation of mesenchymal stromal cells from human placenta, see the "Supplementary methods".

3. Results

3.1. Generation of the IGF1-pLenti6 and the IGF1-pLenti7.3 lentiviral vectors

We constructed and compared two types of lentiviral vectors, the pLenti6 and the pLenti7.3, the latter containing the optimized sequences for Polypurine Tract (cPPT) and Woodchuck Post-transcriptional Regulatory Element (WPRE). The pLenti6 carries a single expression cassette, controlled by the strong constitutive cytomegalovirus (CMV) promoter, comprising the cDNA sequences of the human IGF1 and the GFP genes, spaced by the internal ribosome entry site (IRES) sequence (Fig. 1a). The product of the transcription is a bicistronic mRNA that yields, after translation, two different proteins: the IGF1 and the GFP.

The pLenti7.3 plasmid carries two independent expression cassettes: one expressing the IGF1 cDNA under the control of a CMV promoter, the other expressing the GFP and controlled by the simian vacuolating 40 virus (SV40) promoter (Fig. 1b). To prevent unexpected recombination issues, we preliminarily searched for interspersed repeats of IGF1 using the online software RepeatMasker (http://www.repeatmasker.org/) and we blasted the IGF1 sequence with the plasmid backbone sequence with NucleotideBlast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Table 1). The sequence analysis showed that IGF1 contains 13 bp annealing with pLenti7.3 in the ampicillin resistance gene, that should not affect the recombination reaction, and 11 bp sharing identity with the long terminal repeats (LTR) ΔU3/3′ sequence that theoretically may create problems with gene cloning. Indeed, restriction analy-