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Baculovirus-induced recombinant protein expression in human mesenchymal stromal stem cells: A promoter study

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ABSTRACT

Human mesenchymal stem cells (hMSCs) are the current workhorses of regenerative medicine and gene therapy. The corresponding vectors are usually based on lentiviruses, adenoviruses, retroviruses or adeno-associated viruses, but recently they have been joined by baculoviruses, which are more widely known for their role in the development of pesticides and vaccines. Here we show that gene transfer to an immortalized human mesenchymal stroma cell line can be achieved by baculovirus transduction. We also compared the performance of five different constitutive promoters controlling GFP expression. The human elongation factor 1α promoter (EF1α) achieved the strongest GFP expression, whereas the mouse phosphoglycerate kinase 1 promoter (PGK) was the weakest. The human EF1α promoter is therefore recommended for the regulation of genes introduced into hMSC-TERTs by baculovirus vectors.

Introduction

Human mesenchymal stem/stromal cells (hMSCs) are widely used in regenerative medicine, gene therapy and cell therapy and are regarded as the cell type of choice in the corresponding clinical trials [1–3]. A recent search of the clinical trials database revealed 219 studies including the phrase “human mesenchymal stem cell” and 41 including the phrase “human mesenchymal stromal cell” (www.clinicaltrials.gov accessed Oct, 13th 2016). The popularity of hMSCs reflects the convenience of their isolation from e.g. bone marrow, fat tissue or umbilical cord blood, and their potential for differentiation into e.g. chondrocytes, osteocytes, tenocytes or muscle cells [4,5].

Gene therapy often involves the use of disarmed viral vectors that in their native forms are associated with diseases, e.g. lentiviruses, retroviruses, adenoviruses [6–9]. The drawbacks of such viruses include the need for biosafety level 2 facilities or higher to ensure safe handling, the likelihood of inactivation by pre-existing immunity in the patient, and their potential for genomic integration [10–12].

Autographa californica multicapsid nucleopolyhedrovirus (*Ac*MNPV) is a rod-shaped, double-stranded DNA virus from the baculovirus family that replicates in insects [13] but can also transduce certain vertebrate cell lines, where the genome is maintained as a non-replicating episomal replicon [14]. *Ac*MNPV has been pseudotyped using the vesicular stomatitis virus glycoprotein (VSV-G) to expand its host cell range and prolong recombinant protein expression [15,16]. Further modifications include the insertion of the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), which increases recombinant protein expression [17,18]. Modified baculoviruses offer a powerful alternative to mammalian viruses for gene therapy and regenerative medicine applications because the viral genome is maintained temporarily, neither replicating episomally nor integrating [19–21]. In most cases, the gene of interest (GOI) was placed downstream of the cytomegalovirus major immediate early promoter (CMV), which is provided in the widely-used BacMam Kit (Invitrogen).

It is important to determine promoter characteristics such as the strength and duration of protein expression because these factors influence the success of gene transfer to heterologous cells. However, few comparative promoter studies have considered the wide range of cell types used as targets for gene therapy. The most comprehensive investigation published thus far involved the stable integration of lentiviral vectors into diverse cell lines, and found that the performance of the same promoter varied widely [22]. Furthermore, highly active promoters in mammalian cells, such as the widely-used CMV promoter, are considerably weaker in stem cells, in many cases due to the phenomenon of promoter silencing [23–25]. Promoters should therefore be evaluated in stem cells before they are accepted for use in gene therapy.

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Here we describe the characterization of five promoters in hMSC-TERT cells transduced using baculovirus vectors: the abovementioned CMV promoter, the human elongation factor 1α promoter (EF1α), the mouse phosphoglycerate kinase 1 promoter (PGK), the chicken β-actin promoter coupled with CMV early enhancer (CAGG) and the human ubiquitin C promoter (UBC). The performance of the promoters was compared by analyzing the expression of green fluorescent protein (GFP) by flow cytometry.

Material and methods

Cultivation of Sf9 and hMSC-TERT cells

TriEx™ Sf9 cells (Merck Millipore) were cultivated in Sf-900™ II SFM (Thermo Fisher Scientific) and passaged twice weekly. The inoculum density was 0.5×10^6 c/mL in baffled shaker flasks at 27 °C and 85 rpm (Celltron, Infors HT, Einsbach, Germany).

The immortalized model stem cell line hMSC-TERT [26], kindly provided by M. Kassem (University of Southern Denmark, Odense, Denmark) was cultivated in tissue flasks (Sarstedt, Nümbrecht, Germany) containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 2 mM L-alanyl-L-glutamine (all provided by Biochrom, Berlin, Germany). For regular passaging, cells were seeded at a density of 3000 cells/cm^2 and cultivated at 37 °C in a 5% CO_2 humidified atmosphere until confluence was achieved.

Golden gate cloning

The transfer plasmid was prepared by Golden Gate cloning [27]: 100 ng of each DNA element (VSV-G cassette, mammalian promoter, GFP, polyA signal) and the acceptor vector (containing the Tn7 transposition sites, Fig. 1) were mixed with 2.5 U BsaI, 300 units of 4 DNA ligase, 10 x DNA ligase buffer (all provided by New England Biolabs, Frankfurt, Germany) and topped up to 15 µL with MilliQ water. PCR was carried out in a peqSTAR 2× Universal Gradient thermocycler (Peqlab, Erlangen, Germany) for 25 cycles, each comprising 2 min digestion (37 °C) and 5 min ligation (16 °C) followed by incubation for 10 min at 50 °C and 20 min at 65 °C. The Golden Gate products were stored at 4 °C.

DNA elements

The following elements were used for Golden Gate cloning, listed with their GenBank accession numbers: vesicular stomatitis virus G protein (VSV-G, sequence from vector pHCMV-VSV-G, GenBank accession no. AJ318514.1AJ318514.1, bp: 1420–2955), CMV major immediate early promoter (CMV, sequence from Clontech pEGFP-N1 vector, bp: 1–568), human ubiquitin C promoter (UBC, sequence from dCas9-3xNLS-VP64 vector, GenBank accession no. KJ796484.1KJ796484.1, bp: 2618–3821), mouse phosphoglycerate kinase 1 promoter (PGK, sequence from pTVDll42AEmerald vector, GenBank accession no. KF293661.1KF293661.1, bp: 4098–4612), human elongation factor-1 alpha promoter (EF1α, sequence from pCK25 vector, GenBank accession no. HQ644134.1HQ644134.1, bp: 470–1653), chicken β-actin promoter coupled with CMV early enhancer (CAGG, sequence from vector pTurbo-Cre vector, GenBank accession no. AF334827.1AF334827.1, bp: 1–1718), woodchuck hepatitis virus posttranscriptional regulatory element (WPRE, sequence from Invitrogen pCMV-DEST, bp: 9104–9762). The DNA elements were synthesized by eurofins Genomics (Ebersberg, Germany)

Transformation of DH10Bac cells

MAX Efficiency® DH10Bac™ Competent Cells (Thermo Fisher Scientific) were transformed according to the manufacturer's protocol with the following modifications: 100 ng of each transfer vector was used, and LB medium was used after heat shock instead of SOC medium. Following overnight incubation at 37 °C, positive colonies were picked and streaked onto LB gentamicin, kanamycin sulfate, tetracycline, X-Gal and IPTG plates. Simultaneously, LB liquid cultures with the same antibiotics were inoculated and cultivated overnight. The selected bacmids were isolated as previously described [28]. The samples were incubated at 4 °C rather than on ice and the DNA pellet

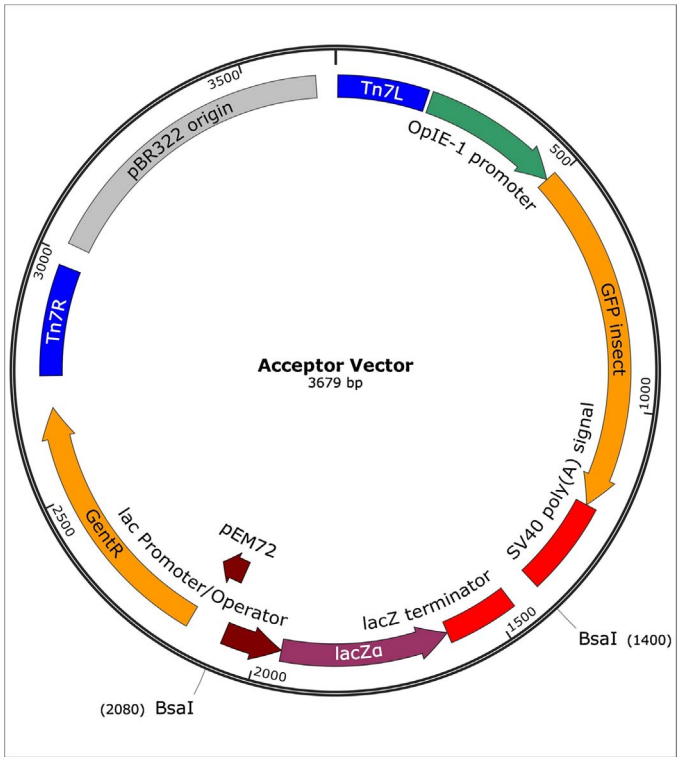

Fig. 1. Plasmid map of the acceptor vector B03 GentR. During the Golden Gate reaction the lacZα cassette is exchanged by the different DNA elements (VSV-G cassette, mammalian promoter, GFP, polyA signal). After transformation of the MAX Efficiency® DH10Bac™ Competent Cells all elements flanked by TN7L and Tn7R are integrated into the Bacmid DNA.