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In vitro expression of erythropoietin by transfected human mesenchymal stromal cells

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Background

Mesenchymal stromal cells (MSC) are pluripotent progenitor cells that can be found in human bone marrow (BM). These cells have low immunogenicity and could suppress alloreactive T-cell responses. In the current study, MSC were tested for their capacity to carry and deliver the erythropoietin (EPO) gene in vitro.

Methods

Expanded BM MSC was transfected with EPO-encoded plasmid pMCV1.2 and EPO-encoded MIDGE (minimalistic immunologically defined gene expression) vector by electroporation. The expressed EPO was used to induce hematopoietic stem cells (HSC) into erythroid colonies.

Results

The results showed that the MIDGE vector was more effective and stable than the plasmid (pMCV1.2) in delivering EPO gene into MSC. The supernatants containing EPO obtained from the transfected cell culture were able to induce the differentiation of HSC into erythroid colonies.

Discussion

MSC hold promise as a cell factory for the production of biologic molecules, and MIDGE vector is more effective and stable than the plasmid in nucleofection involving the EPO gene.

Keywords

erythropoietin, lipofection, mesenchymal stromal cells, minimalistic immunologically defined gene expression (MIDGE), nucleofection, plasmid.

Introduction

Mesenchymal stromal cells (MSC) are adult human pluripotent progenitor cells found in bone marrow (BM). MSC are suitable for manipulation in gene delivery because (1) they are easily isolated and expanded in culture; (2) they are able to maintain an undifferentiated state unless exposed to certain differentiation stimulators and thus can be kept in large volumes for a long period; (c) genetically altered MSC can also be easily recovered after installation *in vivo*; and (d) transduced MSC and their progeny can express newly introduced genes in a less restrictive fashion than other cells, thereby expanding their potential application in treating medical disease. Previous researches have also shown that they have low immunogenicity and even suppress allogeneic T-cell responses [1–4]. Thus allogenic MSC can survive in the microenvironment after *in vivo* transplantation in animal models.

MSC had been used experimentally to carry and deliver numerous therapeutic genes, for example coagulation factor VIII (FVIII) [5], cytotoxic T-lymphocyte associated antigen immunoglobulin (CTLAIg) [6] and α-galactosidase A [7] to treat hemophilia A, graft vs. host disease (GvHD) and Fabry's disease, respectively. Recently MSC have also been shown to have high tumor tropism and were demonstrated to exert an anti-tumor effect and further

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prolong the survival of a rat glioma model when genetically engineered MSC expressing interleukin-2 (IL-2) were injected intratumorally [8,9].

Despite a promising future for gene therapy by manipulating MSC, vector systems for gene therapy strategies should offer both a means of successful transfection and a maximum of safety for the patients. Most gene transfer protocols have used murine replication-defective retroviral vectors or adenoviral vectors. Although retroviral vectors can effectively transduce and integrate into the genome of targeted cells, the risk of oncogene activation has to be considered. Adenoviral vectors have significantly improved transduction efficacy but the death of an 18-year-old patient who received an adenoviral-delivered therapeutic gene in 1999 has raised concern regarding the safety of the therapy using viral vectors [10–12].

Plasmid-based gene transfer using physical or chemical transfection methods avoids these risks. However, transfection efficiency is usually lower and protein expression may not be sustainable for non-viral vectors. There is a substantial risk of immunologic side-effects, including elimination of transfected cells by the host's immune reaction when therapeutically unwanted eu- and prokaryotic proteins (e.g. antibiotic resistance genes, viral protein genes and prokaryotic promoters) are expressed as antigen (Ag) on transfected cells [13]. Moreover, plasmid DNA contains immune stimulatory sequences, called CpG motifs, and they can activate both the innate and acquired arms of immune responses [14,15].

The construct of minimalistic immunologically defined gene expression (MIDGE) has been described previously and has abolished the transfer of therapeutically detrimental sequences [13]. Hence in this study we wanted to determine the capability of MSC to carry and express therapeutic genes using MIDGE as a vector for gene delivery *in vitro* by means of electroporation. The gene we chose for the study was erythropoietin (EPO), which is important in stimulating the production of red blood cells (RBC). The MSC used had been isolated and identified morphologically, cytochemically and immunochemically by flow cytometry. The cells were capable of differentiating into adipocytes, chondrocytes and osteoblasts [16,17].

Methods

Samples

Two samples of human MSC were used in the gene transfer study. The first sample was labeled as pMSC and was isolated from the BM aspirate of a megaloblastic anemia patient, who came for a routine check up at the Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia, after informed consent and under a protocol approved by the UKM Research Committee and Ethics Committee. The second human MSC sample was bought from Cambrex Bio Science Walkersville Inc. (Walkersville, MD, USA) and labeled as hMSC.

Isolation of BM MSC

Five milliliters of BM aspirate were layered on top of 3 mL Ficoll–Pague (Amersham Biosciences, Uppsala, Sweden) and centrifuged at 400 ***g*** for 30 min. The mononuclear cells (MNC) in the interface (density gradient 1.077 g/L) were extracted and washed twice with culture medium by centrifuging at 100 ***g*** for 10 min. The pellet cells were then suspended in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, New York, NY, USA) and the viability of cells counted by hematocytometer and trypan blue staining. The results showed that the percentage of viable cells was 99.7%. The cells were then seeded at a density of 1×10^7 cells in a 25-m^2 plastic flask containing DMEM supplemented with 10% fetal bovine serum (FBS; Gibco). The flask was then incubated in 5% CO_2 in air and monitored daily. Once the cells reached confluency, they were detached with 1 mL 0.25% trypsin–EDTA (Gibco) and replated again into new flasks at a similar cell density. Characterization of DMEM-derived adherent cells was performed by using cells from the third and fourth passages 4–5 weeks after the initial culture [16,17].

Construction of MIDGE-EPO

First-strand cDNA was synthesized by using fetal liver total RNA (Cell Applications Inc., San Diego, CA, USA) as template and oligo dT as synthesis primer. Reverse transcription was performed using SuperScript III RNase H-Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). Polymerase chain reaction (PCR) amplification was then carried out using this first-strand cDNA synthesis product as template and synthetic oligonucleotides upstream containing a *Bam*HI restriction site (5′-GCGAGCTC-CACCATGGGGGTGCACGAATGTCCTGCC-3′) and downstream containing a *Sac*I restriction site (5′-GAGCTCTCATCTGTCCCCTGTCCTGCAGGC-3′) targeted at the 5′-end and 3′-end of the EPO gene in the following PCR reaction: 30 cycles of amplification (94°C,