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Original Article

Acoustic waves improves retroviral transduction in human retinal stem cells

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Abstract

Backgrounds: The plasticity of retinal stem cells (RSCs), a type of cells that can differentiate into neuron cells and photoreceptor cells, endows them with potential therapeutic properties that can be applied to regenerative medicine. Gene modification of these stem cells before trans-differentiation and transplantation enhances their survival and increases their therapeutic function. The different ways to effectively deliver gene into RSCs are still discussed. This study aimed to use the acoustic waves to improve the efficacy of gene delivery for RSCs.
Methods: RSCs were obtained from non-fetal human ocular pigmented ciliary margin tissues. The enhanced green fluorescent protein-encoded murine stem cell retroviruses (MSCV) were prepared and used to infect RSCs. Glass chambers containing RSCs, retroviruses, and various concentrations of polybrene (0, 0.8, 2, 4 and 8 μg/mL) were exposed under 20 or 25 Vp-p ultrasonic standing wave fields (USWF) for 5 min. The percentage of green fluorescent protein positive cells in each sample was calculated and compared to test the efficacy of gene transduction.
Results: Our results showed that the efficiency of gene transduction by MSCV infection was enhanced following the concentration of polybrene and the energy of USWF. The percentage of green fluorescent protein positive cells was significantly higher in chambers that contained 8 μg/mL of polybrene and was exposed to 20Vp-p of USWF for 5 min. In addition, the percentage increased in chambers contained 2, 4 and 8 μg/mL of polybrene when they were exposed to 25Vp-p of USWF. Comparing to those did not treated with ultrasound, the efficiency of retroviral transduction to RSCs increased 4-fold after exposed to USWF for 5 min.
Conclusion: We demonstrated the ability of ultrasound standing waves to improve retroviral transduction into RSCs. We believe that this may be applied to the experimental designs of future studies and may have possible therapeutic uses.

Keywords: Gene transfection; Photoreceptor; Retinal stem cells; Retrovirus; Ultrasound standing waves

1. Introduction

The human retina is composed of ten layers of different cell types. Damage or loss of any of them would result in permanent visual disturbance or blindness.[1,2] Various treatments have been tried to restore vision loss caused by cells damaged in retinal diseases, but the results have been unsatisfactory.[3] Retinal stem cells (RSCs) have the capacity of self-renewal

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and can differentiate into various specific retinal cells. Positive results from stem cell-based therapies have been demonstrated for treating some retinal diseases. MacLaren et al. have taken stem cells at later developmental stages and transplanted them into adult mice with photoreceptor loss, thereby demonstrating that there is a particular time window of development for transplant success in restoring sight to the blind mice.[4] This proof-of-principle breakthrough, transplantation of RSCs that results in photoreceptor cells forming new connections to the mature retina, offers promise for the regenerative treatment of retinal disease.

Transfection of specific genes into stem cells would trigger their transdifferentiation and enhance expression of various trophic factors and crucial proteins from these stem cells. It is an effective way to generate stem cells and improve the suitability for transplant. Gene transduction can be performed either by viral or non-viral approaches. Unfortunately, eukaryotic cells have evolved high barriers to the entry of foreign DNA. Methods to improve the efficacy of gene transduction before stem cell therapy has been widely discussed recently.

Ultrasound-assisted gene transfer (UAGT) has been known as a basis of "sonoporation," in which microbubbles are applied to the cell membrane, destroying areas of the transmembrane to allow macromolecules to enter the cell.[5] There is increasing evidence that exposure of eukaryotic cells to ultrasound of relatively modest intensity, within the range emitted by diagnostic transducers in combination with other viral techniques, can enhance transgenic expression by up to several orders of magnitude over naked DNA alone. In combination with the clinical safety profile of ultrasound, it is suggested that ultrasound-assisted gene delivery has great promise as a novel approach to improve the efficiency of retroviral gene delivery. However, the cavitation effects that occur in a liquid medium with UAGT can cause cell structure to be destroyed as well.

To solve this dilemma, we tried using ultrasonic standing wave fields (USWF) for gene transfer into RGCs in this study. RSCs were exposed to USWF at different exposure times, voltages, and concentration of polybrene. We utilized murine stem cell virus (MSCV) as the gene carrier and examined the effect of USWF on the retroviral gene delivery system.

2. Methods

2.1. Human RSCs

This study was approved by the institutional review board of the Taipei City Hospital. RSCs were collected from non-fetal human sources, with the procedure briefly being described in the following. Strips of the pigmented ciliary margin (PCM) tissues of non-fetal human eyes were separately incubated in Hank's Balanced Salt Solution (HBSS) containing 0.05% trysin for 10 min at 37 °C and were mechanically dissociated with Pasteur pipettes in a Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) medium (Gibcol) at 1:1 ratio. Dissociated cells then were centrifuged at 150 g for 5 min, the enzyme solution was removed, and viable cells were counted by trypan blue exclusion and plated as 5000 cells/200 μL per well in 96-well plates (Corning, Acton, MA) replaced with serum-free culture media composed of DMEM/F-12 medium (Gibcol) with insulin (25 μg/mL; Sigma), transferrin (100 μg/mL; Sigma), progesterone (20 nM; Sigma), putrescine (60 μM; Sigma), sodium selenite (30 nM; Sigma), and human recombinant EGF 20 ng/mL and bFGF 20 ng/mL (R&D Systems, Minneapolis, MN).

The number of primary spheres generated in each well was assessed seven days after plating. The primary suspended spheres were mechanically dissociated into single cells by trituration and an aliquot was counted to determine the total number of cells. For secondary cultures, 500 cells/200 μL per well were plated in each well in 96-well plates using the same culture conditions. The number of secondary spheres in each well in each culture condition was scored seven days after plating. The primary and secondary isolated spheres were plated on poly-L-ornithine-coated (15 μg/mL) glass coverslips in individual wells of 24-well plates (1.0 mL/well) in DMEM/F-12 medium containing 2% fetal calf serum (FCS; Gibcol) and without epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). Coverslips were processed eight days later and fixed with 4% paraformaldehyde for 20 min.[6]

2.2. Reverse transcriptase-polymerase chain reaction (RT-PCR)

For RT-PCR, the total RNA was extracted using the RNeasy kit (Qiagen, Valencia, CA, USA) as described previously.[7] In brief, the first strand of cDNA was synthesized by extending the oligo-dT primer with 25 units of Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT; Promega, Madison, WI, USA) in a mixture containing 1 μg total RNA, 6 mM $MgCl_2$ and 2 mM Deoxynucleotide (dNTP) at 37 °C for 1 h. PCR of the cDNA was performed in a final volume of 50 μl containing 0.2 mM dNTP, 2 mM $MgCl_2$ 20 pmole of opsin primer and 5 μl first strand cDNA. Amplification of β-actin served as the internal-control for sample loading. The primers for RT-PCR were as follows: THf: TTCTGGA-ACGGTACTGTGGCTA (nt 1140-1161, GenBank accession no. L22651), THr: TGGGAGAACTGGGCAAATGT (nt 1397-1416); 5-HTf: AAGAGGGAAGGAGATGGTGGAT (nt 79-100, GenBank accession no. U31884), 5-HTr: AGCC-CAGGAGAAGCCAATGC (nt 354-373); Bcl-2f: TGTCA-CAGAGGGGCTACGAG (nt 302-321, GenBank accession no. L14680), Bcl-2r: GAGCGATGTTGTCCACCAGG (nt 729-748). FGFR1(f), 5'-ATGGCACCCGAGGCAT TATT-3' (nt 2725-2744, GenBank accession no. NM_000604), FGFR1(r), 5′-GGCT CATGAGAGAAGACGGAAT-3' (nt 3125-3104). Reactions were prepared in duplicate and heated to 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 10 min, annealing at 55 °C for 5 min, and extension at 72 °C for 20 min.

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