Nonviral Transfection of Adipose Tissue Stromal Cells: An Experimental Study

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Delivery of plasmid DNA and interfering RNA into adipose tissue stromal cells was carried out by the methods of lipofection, calcium phosphate method, and by electroporation. The percent of transfected cells after delivery of plasmid DNA by the calcium phosphate method and lipofection was 0 and 15%, respectively, *vs.* more than 50% after electroporation. Similar results were obtained for delivery of short-strand RNA into cells. These data indicate that electroporation is the most effective method of nonviral transfection of adipose tissue stromal cells.

Key Words: *adipose tissue stromal cells; transfection; electroporation*

Adipose tissue stromal cells (ATSC) are a heterogeneous population containing mesenchymal progenitor cells [8]. The development of a technology for the use of these cells for cell therapy is in progress. After intravenous injection, ATSC migrate into foci of lesions and hence, can serve as carriers of genetic constructs for local production of therapeutic proteins and growth factors [1]. In addition, ATSC can be regarded as an easily available model for studies of mesenchymal progenitor cells *in vitro* and *in vivo*. The development of these research trends requires effective and available methods for introduction of genetic constructs into these cells for selective suppression of gene expression by RNA interference (specific destruction of mRNA after introduction of complementary antisense RNA oligonucleotides into the cell) and for superexpression of therapeutic growth factors.

MATERIALS AND METHODS

Adipose tissue stromal cells were isolated from mouse subcutaneous fat by the standard method (collagenase 1 treatment) [8]. The cells were cultured in DMEM/10% FBS until passage 2, after which transfection with lipofectamin [4,7], calcium phosphate [3], or electroporation [5,6] was carried out.

Calcium phosphate transfection was carried out as follows. The cells were inoculated to 3.5-cm Petri dishes (200,000/dish) and a mixture of calcium chloride, buffer, and pIRES-GFP plasmid or RNA oligonucleotide solution was added according to manufacturer's protocol (Promega). The precipitates formed after 1 h. The medium with transfection mixture was replaced after 24 h.

For lipofectamin transfection, the cells were inoculated to 3.5-cm Petri dishes (200,000/dish). After 24 h, the medium was replaced for DMEM without serum and antibiotics and the lipofectamin (Lipofectamin 2000, Invitrogen) transfection mixture and plasmid DNA (4 μg) were added. In experimental series II, transfection was carried out using small interfering RNA, SMAD2 complementary genes (SKU 12937-09, Invitrogen), and beclin 1. After 10 h, the medium was replaced for DMEM/10% FBS.

Cell electroporation was carried out according to the protocol developed by Amaxa company. The cells were removed and precipitated for 5 min at

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900 rpm. The precipitate was then resuspended in electroporation buffer, pMAX-GFP plasmid (4 μg) or interfering RNA solution was added, and electroporation was carried out by the T-30 protocol for mouse preadipocyte fibroblasts. The cells were then inoculated into Petri dishes.

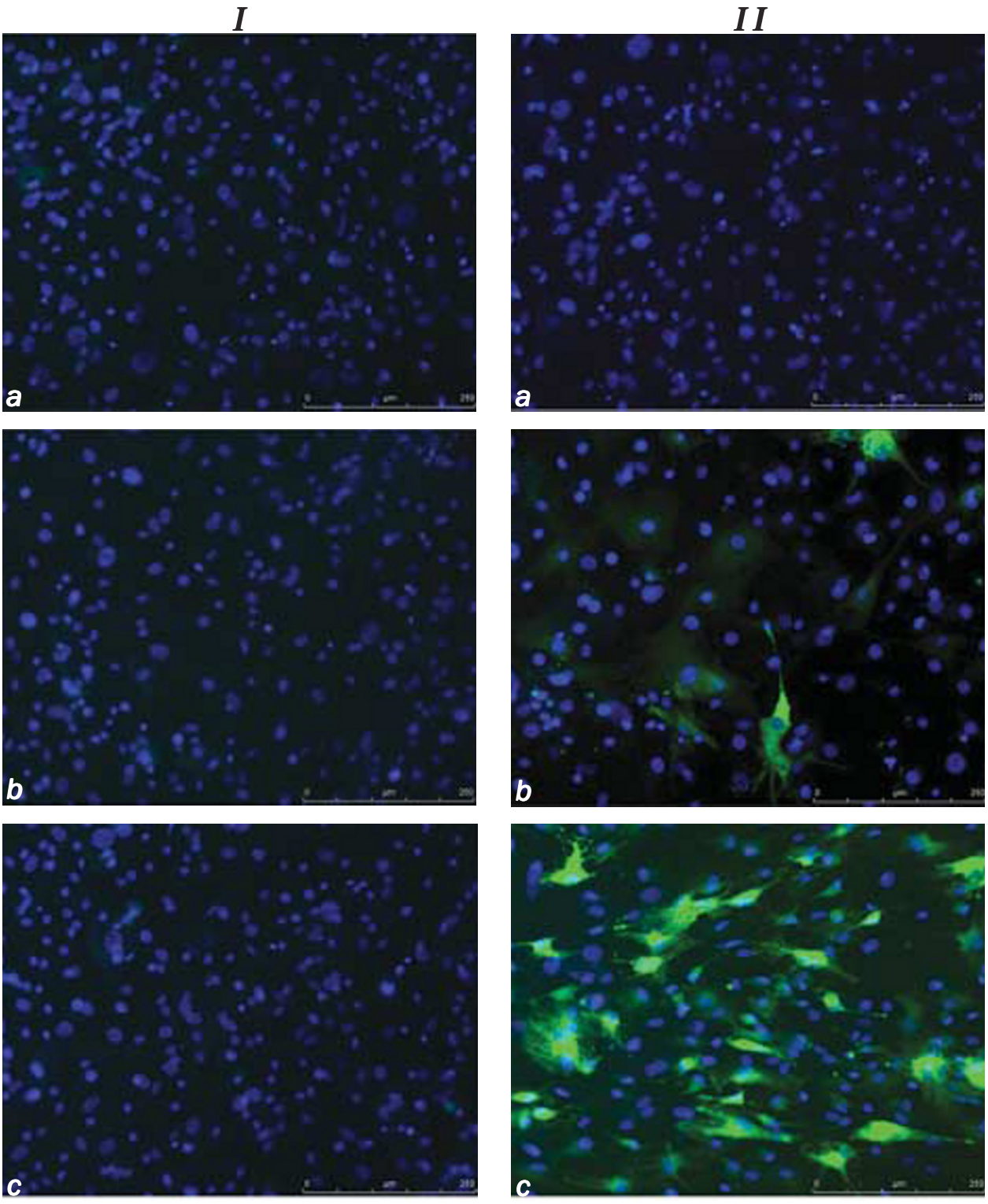

Fig. 1. Mouse FTSC 48 h after transfection: passage 2 ATSC transfected with pMAX-GFP by calcium phosphate method (*a*), lipofectamin (*b*), or electroporation (*c*). Transfected cells contained GFP (green fluorescence). Cell nuclei poststained with DAPI (blue fluorescence). *I*) control ATSC; *II*) transfected ATSC.

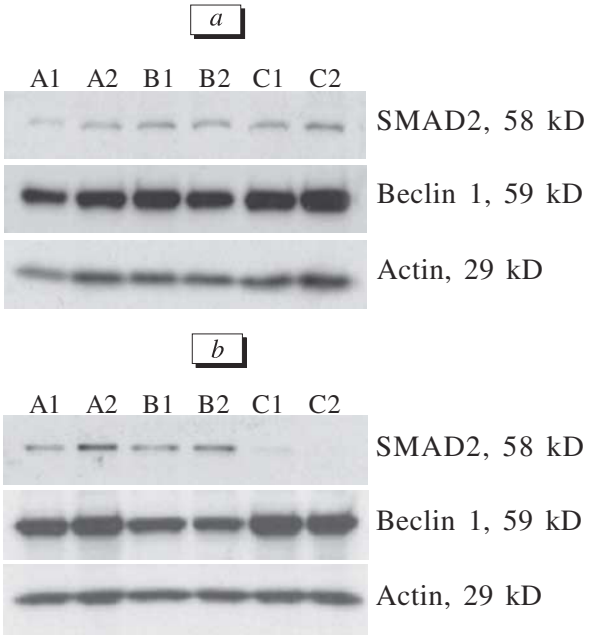

Fig. 2. Content of beclin 1 and SMAD2 in ATSC after RNA interference: passage 2 ATSC transfected by short-strand RNA complementary to beclin 1 or SMAD2 mRNA by lipofectamin (*a*) and electroporation (*b*). Tracks: A1 and A2: ATSC transfected by control RNA 24 and 72 h after transfection; B1 and B2: ATSC transfected by anti-beclin 1 RNA 24 and 72 h after transfection; C1 and C2: ATSC transfected by anti-SMAD-2 RNA 24 and 72 h after transfection.

Total RNA was isolated from cells using Qiagen RNeasy Mini Kit by the method described in manufacturer's instruction. Genome DNA was isolated from the specimens using RNase Free DNase Set (Qiagen). The concentration of RNA was evaluated by absorption of RNA solution at λ=260 nm. The content of the product in each sample was standardized by the content of mRNA amplification products of house keepings, GAPDH, and actin genes. The following pairs of primers were selected for quantitative real-time PCR: SMAD2 Fw 5' ATGTCGTCCATCTTGCCATTC 3' Rv AACCGT CCTGTTTTCTTTAGCTT 3', amplicon length 173 b. p.; GAPDH Fw 5' GACCCCTTCATTGACCTC AACTAC 3' Rv 5' TGGTGGTGCAGGATGCATT GCTGA 3', amplicon length 361 b. p.; actin Fw 5' AGTGTGACGTTGACATCCGTA3', Rv 5' GCCAG AGCAGTAATCTCCTTCT 3'.

The reduction in the content of target gene products in RNA interference was analyzed by Western blot [1]. The equivalence of the sample application was standardized by the content of actin. The following reagents were used: polyclonal rabbit antibodies to SMAD2 (Abcam), beclin 1, and actin (Santa Cruz, primary antibodies); antibodies to rabbit IgG, conjugated with horseradish peroxidase (second antibodies); enhanced chemiluminescence immunoblot development kit (Amersham). Quantitative analysis was carried out after computer scanning of images using Scion Image software, ImageJ version (Scion Inc.).

The efficiency of nonviral transfection methods for delivery of plasmid DNA in ATSC was evaluated using pMaxGFP (Amaxa) carrying green fluorescent protein (GFP) complementary DNA (cDNA). The efficiency of transfection was evaluated after 24, 48, and 72 h by counting GFP-labeled cells. Reduction of gene expression in RNA interference was verified by real-time PCR at the level of mRNA and by Western blot method at the level of protein.

RESULTS

Relationship between transfection methods and ATSC viability. High toxicity of nonviral transfection is a limitation of this method. We analyzed cell viability directly and 24 h after transfection. Trypan blue staining of cells incubated in transfection mixtures showed that the percentage of viable cells after calcium phosphate transfection was 98±2%, after lipofection 92±7%, and after electroporation 72±12%. Hence, these methods for nonviral transfection did not modify ATSC viability.

Transfection efficiency. No expression of GFP was detected 24, 48, or 72 h after calcium phosphate transfection of ATSC. The percentage of GFP-containing cells after lipofectamin transfection did not exceed 5% after 24 h and 16±9% of all cells after 48 and 72 h. The percentage of GFP-containing cells 24 h after electroporation of ATSC was 50±14% and later reached 85% (after 1 week; Fig. 1). After 2 weeks of culturing, more than 10% cells steadily expressed GFP.

The efficiency of nonviral transfection methods under conditions of selective suppression of gene expression by RNA interference was evaluated using short-strand RNA fragments complementary to SMAD2 and beclin 1 mRNA. Introduction of these short-strand RNA into cells caused selective degradation of the complementary mRNA. Transfection efficiency was evaluated after 24, 48, and 72 h by reduction of mRNA and protein levels by real-time PCR and immunoblotting.

Since we revealed extremely low efficiency of calcium phosphate transfection, we did not use this method of cell transfection by short-strand RNA. After lipofectamin transfection, the levels of mRNA of the studied genes (SMAD2, beclin 1) decreased on day 2, but the levels of some control genes and house keepings genes mRNA decreased in parallel with it (Fig. 2, *a*). mRNA levels for many genes

changed 24 h after electroporation, but after 48 and 72 h the total transcription level was restored, while the content of target gene mRNA decreased. The specificity of the expression suppression was confirmed by Western blot. The content of products of exclusively suppressed genes decreased 24 and 72 h after electroporation (Fig. 2, *b*).

Hence, ATSC transfection by lipofectamin or calcium phosphate does not reduce viability of these cells, but fails to effectively deliver the genetic material. In contrast to these to methods, electroporation reduces cell viability by 17%, but provides effective delivery of plasmid DNA and short-strand RNA. Presumably, membrane depolarization caused by electric pulse ensures high penetration of the genetic material into the cell. This, in turn, causes stable expression of the delivered gene or specific RNA interference. These data indicate that electroporation is the most effective method of nonviral transfection of ATSC.

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