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Core-shell nanoparticle controlled hATSCs neurogenesis for neuropathic pain therapy

Jee In Choi [1], Hyun Tae Cho [1], Min Ki Jee, Soo Kyung Kang*

Laboratory of Stem Cell Biology, Department of Biotechnology, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

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ABSTRACT

A stem cell-based strategy for tissue engineering in regenerative medicine is crucial to produce and effective therapeutic replacement of injured or damaged tissues. This type of therapeutic replacement requires interaction with the cells and tissues via the incorporation of a beneficial physical microenvironment and cellular biochemical signals. Recently, we studied a cell-function modifying factor, core-shell nanoparticles consisting of an SPIO (superparamagnetic iron oxide) core covered with a photonic ZnO shell for human adipose tissue-derived stem cells (hATSCs) that regulate various cellular functions: self-renewal, neurogenesis, and dedifferentiation. We proposed an alternative method of stem cell culture that focuses on the use of Zn++ Finger nanoparticles for stem cell expansion and trans-differentiation modulation *in vitro* and in *in vivo* spinal cord injury models. Our study showed that treating hATSC cultures with nanoscale particles could lead to active cell proliferation and self-renewal and could promote nuclear Dicer-regulation of several functional molecules, Oct4 and Glutathione peroxidase 3 (GPx3), and the abundance of specific functional proteins that have been observed using biochemical analysis. These biochemical changes in hATSCs induced the functional development of multiple differentiation potencies such as β-cells and neural cells; specifically, the ability to differentiation into GABA-secreting cells was significantly improved in *in vitro*- and *in vivo*-induced animal lesions with significantly improved therapeutic modality.

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1. Introduction

Stem cells, including bone marrow, fat, or other tissue-derived cells, are considered multipotent and normally only have the ability to replenish specific cells or tissues in therapeutic applications [1]. However, autologously available fat or bone marrow-derived stem cells have limited self-renewal and ability to transdifferentiate into neural cells, β-cells, hematopoietic cells, etc. Previously, we developed a cell-based biological system involving human ATSCs (adipose tissue stromal cells), which have the potential to differentiate into multiple cell lineages including neurons [1–3]. Tissue-specific stem cells are currently being investigated for the ability of a given cell type to reciprocally dedifferentiate, redifferentiate, and/or transdifferentiate in response to specific stimuli, including biochemical and physical stimuli [4,5]. Our results have revealed that adult tissue-specific stem cells have the capacity to participate in regeneration after transdifferentiation into various lineages of cells or tissues. Our data also suggest that the limited transdifferentiation potency of adult stem cells is reversible and that these cells can be transdifferentiated via dedifferentiation and self-renewal processes that include cell reprogramming. Data is not shown, but it is supposed that human ATSCs are reprogrammed into more primitive stem cells after the ectopic induction of IPSC (induced pluripotent stem cell) factor expression, either directly or indirectly via an increase in their proliferation and differentiation, into functional tissues or cells. Transcription factors that control stem cell plasticity and self-renewal have been identified [6,7], and the core regulatory circuitry by which these factors exert their regulatory effects on protein coding genes has been described [8–13]. Although adult somatic stem cells possess varying lifespans, it is possible to extend their life cycles via gene expression regulation, which suggests that they might be useful as an autologously available therapeutic cell source.

* Corresponding author. Laboratory of Stem Cell Biology, Department of Biotechnology, School of Veterinary Medicine, Seoul National University, 599 Gwanangno, Gwanak-gu, Seoul 151-742, Republic of Korea. Tel.: +82 02 880 1247 (office), +82 010 3634 7422 (mobile).
E-mail address: sookang@snu.ac.kr (S. Kyung Kang).
[1] These authors were equally contributed.

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Recent studies have presented evidence that stem cells are highly sensitive to their microenvironment [14–16] and that stem cells directly respond to microenvironmental cues such as the stiffness of a substrate in two and three dimensional (3D) culture and topography [17,18]. Stem cells may encounter topographies with different pore sizes (from macro to micro), arrangements, and morphologies and projections of other cells. Possibly, nanoparticle was infiltrated into cytosolic region of stem cells and affected on protein conformation and expression via the component of nanoparticle bioactive domain, Zn++ Finger. Matrix and microenvironment have the potential to influence cell behavior and functionality. Interaction with nanotopographies can alter cell structure, adhesion, and mobilization. Cell growth and proliferation can significantly affect protein expression or phosphorylation via gene expression regulatory pathways. The use of biomaterials, including nanoparticles, has an advantage over the use of defined media that facilitate cell growth and differentiation into specific lineages for application in regenerative medicine. In the field of tissue engineering, the use of stem cells has the potential to produce patient-specific tissues or cells that do not carry the risk of immune rejection. The primary goals in the application of nanoparticle-containing biomaterials during stem cell culture are to provide cues that effectively guide the differentiation of stem cells into a specific lineage of mature cells or tissues and to allow the stem cell to maintain undifferentiated, actively self-renewing cells. Nano materials must possess at least one dimension in the nanometer scale and also an SPIO core covered with a photonic ZnO shell effectively interacts with functional transcription factor including Dicer, Oct4, Sox2, Nanog, and also GPx3. Those functional factors were basically and commonly attractive to ZnO core-shell structure and enhances noncovalent binding for facilitating catalytic function of those functional proteins directly. Biophysical properties of SPIO core was covered with a photonic ZnO core-shell and was effectively contributed and activated biochemical function of Dicer, Oct4, Sox2, Nanog, and GPx3 for functional improvement of stem cell. Finally, improved differentiation ability of stem cell expanded a potential application in the fields of stem cells biology and regenerative medicine. Particularly, nanoparticles have become highly valuable tools in the biological sciences because of their biocompatibility, facial conjugation to biomolecules, and tunable optical properties depending on size, surface, shape, and characters. Recently, various types of nanoparticles have demonstrated applicability in gene and drug delivery, vector transfection, and as a DNA binding agent for gene therapy.

In this study, the functional modality of core-shell nanoparticles consisting of an SPIO core covered with a photonic ZnO shell was effectively facilitates regenerative activity of human adipose-derived stem cells (hATSCs). Under the molecular control of core-shell particles, nuclear-localized Dicer leading to hATSCs multilineage differentiation and will contributes regenerative medicine in the central nervous system disorder via a stemness-mediated proper cell reprogramming process.

2. Materials and methods

2.1. Isolation and culture of adipose tissue-derived stem cells

Donor-derived the raw adipose tissues were processed in accordance with established methodologies in order to determine the stem vascular functions. Human raw fat tissue obtained from the patient abdomen (as following patient's approval document) was processed according to established methodologies to determine stem cell vascular function. In order to isolate the stem cells, the samples were digested with 0.075% collagenase IV (Sigma) and centrifuged at 1200× *g* for 10 min to acquire a high density cell pellet. The pellet was then suspended in red blood cell (RBC) lysis buffer (Biowhittaker, Walkersville, MD, USA) and incubated for 10 min at room temperature to lyses the contaminating RBCs. The stem cell pellet was then collected and incubated overnight at 37 °C/5% CO_2 in 10% FBS containing α-MEM medium (GIBCO BRL, CA, USA). This work was approved by Seoul National University Institutional Review Board (IRB No. 0603/001-002) and the ethics committee specifically approved that procedure.

2.2. Cell survival and proliferation assay

Cell viability was assessed by visual cell counts in conjunction with trypan blue exclusion. Mitochondrial activity was assessed by measuring the ability of the cortical cultures to reduce 3,4,5-dimethyl thiazol-2-yl-2,5-diphenyl tetrazolium bromide (MTT; Sigma, St. Louis, MO, USA) to a colored formazan using a plate reader. In all viability assays, triplicate wells were established under each experimental condition, and each experiment was repeated at least three times. The raw data from each experiment were analyzed via analysis of variance with Fisher's or *t*-tests. For flow cytometric analysis, cells were cultured in 100-mm dishes at densities that ensured exponential growth at the time of harvesting. Harvesting and processing protocols were used to detect DNA via flow cytometry with propidium iodide. The cells were analyzed with a BD Biosciences FACScan system (San Jose, CA, USA). The percentages of cells in the G0/G1, S, and G2/M phases of the cell cycle were determined using a DNA histogram fitting program (MODFIT; Verity Software, Topsham, ME, USA). A minimum of 10^4 events/samples was collected [19].

2.3. Core-shell nanoparticles treatment in cultured hATSCs

Human ATSC were cultured upto passage 10–13. The hATSC were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 with α-MEM containing 10% FBS and 1% PS (all obtained from Gibco BRL). In present study, we used core-shell nanoparticles obtained from Dr Seong SY from Medical School of Seoul National University. Before treatment of nanoparticles in hATSCs, nanoparticles were collected using magnetic and aliquot in 10 μl α-MEM medium included 300 ng nanoparticles. The hATSCs cultured in 12 well plate achieved 70% confluence per well. After one day, we treated mixture containing 100 ng/ml nanoparticles in culture medium per well. And then the medium was replaced at 48 h.

2.4. Dicer overexpression transfection

For overexpression of Dicer gene in cultured hATSCs, the DICER1 vector (OriGene, NO. SC309637) was transfected into hATSCs using Lipofectamine™ LTX and Plus™ Reagent, referring to the manufacturer's protocol (Invitrogen). Cells were seeded in 12-well plates for one day in order to achieve 70% confluence per well at the time of transfection. 0.6ug of DNA was mixes with DMEM respectively. 5 μl of Lipofectamine in DMEM was added to diluted DNA and incubated for 30 min at room temperature. And then the DNA and Lipofectamine mixture was added in the cells with serum free DMEM. After transfection for 5 h at 37 °C, complexes were removed and the medium was replaced with DMEM containing 10% FBS. And cells were cultured with 10% FBS DMEM medium at 37 °C for 24–48 h transfection. Control group of cells was set with DICER1 gene free vector transfection.

2.5. Real time reverse transcription polymerase chain reaction

Total cellular RNA was isolated with Trizol (Life Technologies, Frederick, MA, USA) and reverse transcribed into cDNA using an oligo-dT primer amplified by 35 cycles (94 °C for 1 min; 55 °C for 1 min; and 72 °C for 1 min per cycle) of PCR using 25 pM of specific primers. The ABI 7700 Prism Sequence Detection System and SYBER green detection kit (Applied Biosystems, Foster, CA, USA) were used to conduct the PCR reactions. The primer was designed with Primer Express software (PE-Applied Biosystems, Warrington, UK) using gene sequences obtained from the GeneBank database. For PCR product labeling, we used a Syber green detection kit purchased from Applied Biosystems.

2.6. Immunofluorescence analysis

Immunofluorescence analysis on the 12 well plate was conducted. Cells were washed with PBS and fixed by incubating cells in a 4% paraformaldehyde (PFA) fixative solution for 20 min at room temperature. After washing with PBS for 10 min 3 times, the cells were blocked for 1 h at room temperature with 1% normal goat serum. And 12 well plate was incubated with primary antibodies overnight at 4 °C. After repetitive washing with PBS, the cells were incubated with Alexa Flour 488 or Alexa Flour 594 conjugated secondary antibodies for 5–10 min. After repetitive washing with PBS, cells were incubated with 40, 6-diamidino-2-phenylindole (DAPI) for counterstaining the nucleus. Finally, cells were mounted using Aqua poly/mount solution (Polysciences, Inc., Warrington, PA) for confocal laser scanning microscopy (Leica Microsystems) analysis.

2.7. Preparation of tissue whole extracts and western blot

For the confirmation of differentially expressed proteins following the de-ATSC, the cultured cells were pooled and lysed in 500 μl of lysis buffer (20 mM Tris–HCl [pH 7.5], 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM EGTA, 1 mM glycerophosphate, 1 mM Na_3VO_4, and 1 mM PMSF). The lysates were clarified via 10 min of centrifugation at 15,000× *g* and the total protein

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