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Co-culture with neurotrophic factor secreting cells induced from adipose-derived stem cells: Promotes neurogenic differentiation

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ABSTRACT

Adipose-derived stem cells (ADSCs) and bone marrow stem cells (BMSCs) can be equally proper in the treatment of neurodegenerative diseases. However, ADSCs have practical benefits. In this study, we attempted to induce the secretion of neurotrophic factors (NTF) in human ADSCs. We then evaluated the effects of co-culture with NTF secreting cells in neural differentiation of human ADSCs. Isolated human ADSCs were induced to neurotrophic factors secreting cells. To evaluate the *in vitro* effects of NTF-secreting ADSCs on neurogenic differentiation of ADSCs, we used neurogenic induction medium (control group), the combination of neurogenic medium and conditioned medium, or co-cultured NTF-secreting ADSCs which were encapsulated in alginate beads (co-culture) for 7 days. ELISA showed increased (by about 5 times) release of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in NTF-secreting ADSCs compared to human ADSCs. Real time RT-PCR analysis revealed that NTF-secreting ADSCs highly expressed NGF and BDNF. In addition, co-culture with NTF-secreting ADSCs could also promote neuronal differentiation relative to gliogenesis. Overall, NTF-secreting ADSCs secrete a range of growth factors whose levels in culture could promote neuronal differentiation and could support the survival and regeneration in a variety of neurodegenerative diseases.

1. Introduction

Mesenchymal stem cell (MSC)-based therapies may be efficient in the treatment of neurological diseases. The efficiency of MSCs as a clinical therapeutic implement for neurodegenerative diseases has been recently investigated in two points, transdifferentiation into neural cells and replacing damaged cells [1] or production of cytokines as trophic factors for support and regeneration of damaged nervous tissue [2–4].

Bone marrow stromal cells (BMSCs) were the first type of adult stem cells to successfully differentiate into neuronal cells and promote neuronal survival in cerebral ischemia [2,5]. They have also been used in the functional recovery following spinal cord injury [6]. So; transplantation of MSCs can be enhancing regeneration of damaged neural tissues through secretion of cytokines and trophic factors [7].

Recent evidence suggests that the neurotrophic and neuroprotective effects of induced BMSCs can enhance survival and regeneration in a variety of neurodegenerative diseases [8–12].

However, BMSC harvest is an invasive and painful procedure that might yield few MSCs [13]. Therefore, an alternative cell source is preferred. Unlike bone marrow, adipose tissue is abundant and easily accessible. Moreover, its harvesting does not require any invasive and painful procedures [14].

Adipose-derived stem cells (ADSCs) with similar characteristics can successfully differentiate into chondrocytes, adipocytes, osteoblasts, myocytes, and neuronal linage [15–17]. In addition, compared to BMSCs, a higher proportion of ADSCs express nestin which is a marker of progenitor neural cells [18]. Several bioactive protein factors secreted by ADSCs have been identified [19]. ADSCs secrete a range of growth factors whose levels achieved in culture provided significant protection to neurons [20–23]. Zhao et al. reported that ADSCs possessed potent neuroprotective activity against neuronal excitotoxicity [24]. The protective effect of ADSCs was found to be highly dependent on the presence of brain-derived neurotrophic factor (BDNF), an important neurotrophic factor which prevents neuronal degeneration and is involved in neuronal development [25].

Moreover, conditioned media of MSCs culture have induced neuritogenesis in PC12 cells as well as neuroprotective effects against neurotoxicity agents via neurotrophin contains [24–26].

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However, only selected molecules have been dosed in ADSCs as possible neurotrophic candidates [27,28].

We hypothesized that human ADSCs act as source of variety of neurotrophic factors and can release more neurotrophic factors after induction. Therefore, we decided to induce human ADSCs to secret neurotrophic factors. In this study, we attempted to induce NTF secretion in human ADSCs. We then evaluated the effects of co-culture with NTF secreting cells on neural differentiation of human ADSCs.

2. Materials and methods

2.1. Preparation and culture of human MSCs

All chemicals, except where specified otherwise, were purchased from Sigma–Aldrich (St. Louis, MO). Human adipose tissue was obtained from lipoaspirate samples of abdominal fat from female donors (age range: 23–41 years old) who had provided informed consent. The tissue was cultured as described previously [29]. The cells used in the present study were from passages 3–6.

Flow cytometry analysis of the isolated cells was carried out as previously described [30].

2.2. Induction of human ADSCs into NTF secreting cells

The induction of human ADSCs into NTF secreting cells was carried out according to a previously described method [9]. We used glial fibrillary acidic protein (GFAP) and S100β to confirm the effectiveness of human ADSCs differentiation into NTF secreting cells. In order to detect the ability of the induced cells to secrete neurotrophin, CTNF, GDNF, NGF, and BDNF were assessed by real-time RT-PCR and ELISA.

We evaluated the *in vitro* effects of NTF-secreting ADSCs on neurogenic differentiation of ADSCs using neurogenic induction medium (control group), the combination of neurogenic medium and conditioned medium (CM-NTF) or co-cultured NTF-secreting ADSCs which were encapsulated in alginate bead (co-culture) for 7 days. Nestin, MAP2, and GFAP were used as markers to confirm neurogenic differentiation of human ADSCs using both immunostaining and real time reverse transcriptase PCR analysis.

2.3. Preparation of NTF-secreting ADSCs conditioned medium

Confluent NTF-secreting ADSCs were cultured for 3 days with no serum. The media was then removed and stored at −70 °C prior to use in culture experiments.

2.4. Neurogenic differentiation of human ADSCs

Human ADSCs were dissociated with 0.25% trypsin/0.02% EDTA. They were plated on low-attachment plastic tissue culture plates in DMEM, 2% B27, 20 ng/ml basic fibroblast growth factor (bFGF) and 20 ng/ml hEGF with the formation of floating bodies. The cells were treated with aforementioned factors every 2 days up to 7 days. The spheres were then dissociated and became single by pipetting in trypsin/EDTA. After centrifugation, the cell pellet was cultured in neurobasal medium containing 5% FBS, 1% L-glutamine, 1% non-essential amino acids, 2% N_2 supplement, and 2% B27 for 1 week.

2.5. Preparation of encapsulated alginate beads containing NTF-secreting ADSCs

Alginate contains a natural linear polymer of 1,4-linked β-D-mannuronic acid (M) and α-glucuronic acid (G) with various compositional and sequential structures [31]. Such composition makes it a suitable hydrogel for cell microencapsulation.

Cell encapsulation was carried out by mixing a pellet of NTF-secreting ADSCs with alginate (50:50) and keeping it in a 0.1 M calcium chloride solution for 10 min. The encapsulation yielded beads with a final concentration of 1,000,000 cells/mL in 1% w:v alginate (approximately 20,000 cells per bead). Beads were cultured in 24-well plates containing 20,000 human ADSCs. They were allowed to differentiate in the presence of neurobasal medium supplemented with 1% pen/strep, 1% non-essential amino acids, 2 mM L-glutamine, 2% B27, and 2% N2 for 7 days.

In order to compare the effects of conditioned media of NTF-secreting ADSCs and NTF-secreting ADSCs on enhancing neurogenic differentiation, we considered 3 groups. In group 1, neurogenic differentiation of human ADSCs was performed in neurobasal medium containing 1% pen/strep, 1% non-essential amino acids, 2 mM L-glutamine, 2% B27, and 2% N2. In group 2, neurogenic differentiation of human ADSCs was conducted in the neurogenic medium and conditioned media of NTF-secreting ADSCs (1:1 v/v). In group 3, neurogenic differentiation of human ADSCs was carried out in neurogenic medium which co-cultured with 3 NTF-secreting ADSCs encapsulated alginate beads for 7 days (2×10^4 of NTF-secreting ADSCs encapsulated in each alginate bead). After 7 days, the beads that contained NTF-secreting ADSCs were removed and differentiated ADSCs were assessed for neural markers using immunocytochemical and real-time PCR.

2.6. MTT assay

To examine the viability of NTF-secreting cells after induction, 5 mg of MTT was dissolved in 1 ml of PBS. The stock solution was added to the culture medium at a dilution of 1:10. The plates were incubated at 37 °C for 4 h. The medium was then aspirated and 200 μl of dimethyl sulfoxide (DMSO) was added to extract the MTT formazan. The absorbance of each well was detected by a microplate reader (Hiperion MPR 4+, Germany) at the wavelength of 540 nm.

2.7. Immunocytochemistry

After fixation with 4% paraformaldehyde (PFA)/PBS, cells were treated with blocking solution (PBS containing 4% goat serum and 0.1% Triton X-100) for 45 min at RT. Then, cells were incubated in primary antibodies in PBS/0.1% Triton X-100 and 1% goat serum overnight at 4 °C. Anti-GFAP (1:300; Abcam, UK) and anti-S100β (1:500; Abcam, UK), anti-Nestin (1:300, Abcam, UK), mouse anti-microtubule-associated protein (MAP2, MAP2 and 1:300, Abcam, UK), mouse anti-glial fibrillary acidic protein (GFAP, 1:300, Abcam, UK) were used. After washing with PBS, the slides were exposed to secondary antibodies, i.e. rabbit anti-mouse fluorescein isothiocyanate (FITC) (1:500; Abcam, UK) and rabbit anti-mouse phycoerythrin (PE) (1:200; Abcam, UK)-conjugated. They were incubated at room temperature for 1 h. Diamidino-2-phenylindole (DAPI) was used for nuclear counterstaining. For negative controls, primary antibody was omitted from the reaction series in each experiment. Cells were observed using a fluorescence microscope (Olympus BX51, Japan). To perform quantitative analysis, the numbers of positive cells were counted on each acquired image by ImageJ1.42 (NIH), and the ratio to the number of nuclei was analyzed for each antigen. The number of immunopositive cells was counted in a minimum total of 200 cells per slide. All immunocytochemical experiments were repeated twice.