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Research paper

A new method to effectively and rapidly generate neurons from SH-SY5Y cells

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HIGHLIGHTS

- CM-hNSCs significantly increased the neuronal percent of RA-pretreated SH-SY5Y cells.
- CM-hNSCs with RA significantly shorten the time of neuronal differentiation of SH-SY5Y cells.
- CM-hNSCs significantly inhibited the apoptosis of differentiated SH-SY5Y cells due to the presence of RA.
- CM-hNSCs significantly promote the length of neurite of RA-pretreated SH-SY5Y cells.

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ABSTRACT

It is well known that neurons differentiated from SH-SY5Y cells can serve as cell models for neuroscience research; i.e., neurotoxicity and tolerance to morphine in vitro. To differentiate SH-SY5Y cells into neurons, RA (retinoic acid) is commonly used to produce the inductive effect. However, the percentage of neuronal cells produced from SH-SY5Y cells is low, either from the use of RA treatment alone or from the combined application of RA and other chemicals. In the current study, we used CM-hNSCs (conditioned medium of human neural stem cells) as the combinational inducer with RA to prompt neuronal differentiation of SH-SY5Y cells. We found that neuronal differentiation was improved and that neurons were greatly increased in the differentiated SH-SY5Y cells using a combined treatment of CM-hNSCs and RA compared to RA treatment alone. The neuronal percentage was higher than 80% (about 88%) on the 3rd day and about 91% on the 7th day examined after a combined treatment with CM-hNSCs and RA. Cell maturation and neurite growth of these neuronal cells were also improved. In addition, the use of CM-hNSCs inhibited the apoptosis of RA-treated SH-SY5Y cells in culture. We are the first to report the use of CM-hNSCs in combination with RA to induce neuronal differentiation of RA-treated SH-SY5Y cells. Our method can rapidly and effectively promote the neuronal production of SH-SY5Y cells in culture conditions.

1. Introduction

Neurons differentiated from SH-SY5Y neuroblastoma cells by RA (retinoic acid) treatment are widely used as *in vitro* cell models in neuroscience research topics including neurotoxicity and tolerance to morphine [4,5,9,12,17,19,20]. SH-SY5Y is a human-derived cell line, with the capacity to expand in culture prior to differentiation. In the literature, RA is the most commonly implemented and best-characterized agent to differentiate SH-SY5Y cells into neurons in vitro. However, only about 20% of SH-SY5Y cells can be differentiated into neurons with RA treatment alone [15]. In addition, it takes time (an average of 7 days) to produce a neuronal differentiation of SH-SY5Y cells with RA treatment alone [5,16]. Recently, some investigators proposed that other chemicals (i.e., herbimycin A (herb-A), 12-*O*-tetradecanoyl-phorbol-13

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acetate (TPA), and dibutyryl cyclic AMP [dbcAMP]) or neurotrophic factors such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) with or without extra cellular matrix (ECM) gel, can further improve neuronal differentiation and maintain these conditions in cultures of RA-treated SH-SY5Y cells [3,6,10,15]. Cultivating SH-SY5Y cells in primary neuronal cultures or NSC (neural stem cell) culture medium with a B27 supplement has been shown to enhance neuronal differentiation with RA treatment [17]. However, the percentage of neuronal cells in the differentiated SH-SY5Y cells and the time needed to induce neuronal differentiation remain unsatisfactory under these combined conditions. Furthermore, these methods place an increased financial burden on experimental expenses. Thus, it is necessary to find an effective method to increase neuronal differentiation and production and at the same time to reduce the neuronal differentiation time of SH-SY5Y cells, thus making differentiated SH-SY5Y cells an ideal cell model.

NSCs (neural stem cells) have been successfully isolated from human brain tissue and can be expanded in vitro for an extended time in neural basal culture medium supplemented with B27 and mitotic factors bFGF (basic fibroblast growth factor) and EGF (epidermal growth factor) [18]. Human NSC (hNSC) lines are currently commercially available for research use. It has been reported that NSCs can secrete many neurotrophic factors, such as BDNF, NGF, GDNF (glial cell line-derived neurotrophic factor), and NT-3 (neurotrophin-3), as well as other soluble factors in culture conditions [2,8,13]. Thus, CM-NSCs (conditioned medium collected from hNSC cultures) containing these already known neurotrophic factors may also contain unknown factors released by hNSCs. In previous studies, our group successfully generated self-renewable functional NSC-like cells from hBM-MSCs (human bone marrow-derived mesenchymal stem cells) cultivated in CM-NSCs [7,14], demonstrating that CM-NSCs plays a crucial role in promoting the neuronal differentiation of these connective tissue cells. We hypothesized that CM-NSCs used to culture SH-SY5Y cells with an RA combination may increase neuronal differentiation and production of these cells.

2. Materials and methods

2.1. Collection of CM-hNSCs

hNSCs were purchased from Lonza (Walkersville, MD, USA). The detailed method for the growth and maintenance of undifferentiated hNSC spheroids and for collecting CM-hNSCs was performed according to the protocol we established previously [14]. In brief, hNSCs were cultured in a T-75 flask containing 15 ml complete culture medium including DMEM/F12 (Invitrogen, CA, USA), human recombinant epidermal growth factor (EGF; 20 ng/ml) and basic fibroblast growth factor (bFGF; 20 ng/ml) (R&D Systems, Minneapolis, MN, USA), B27 (serum-free medium supplements formulated to provide optimal growth condition for NSC expansion, 1: 50; Invitrogen), heparin (5 μg/ml; Sigma, St Louis, MO, USA), 2 mM L-glutamine, and an antibiotic–antimycotic mixture (1: 100; Invitrogen) at 37 °C in a 5% CO_2 humidified incubation chamber (Fisher, Pittsburgh, PA, USA). Fifty percent of the culture medium was exchanged with fresh culture medium every 3 days. Larger neuro-spheroids were cut into small spheroids observed under a microscope (Olympus, Japan) every 2 weeks. At each medium change, CM-hNSCs were collected and filtered through a membrane with a 0.22 μm in diameter pore size (Millipore, Billerica, MA, USA). The filtered conditioned mediums were centrifuged at 1000 RPM × 10 min at RT (room temperature) and observed under a microscope to make sure there was no cell contamination.

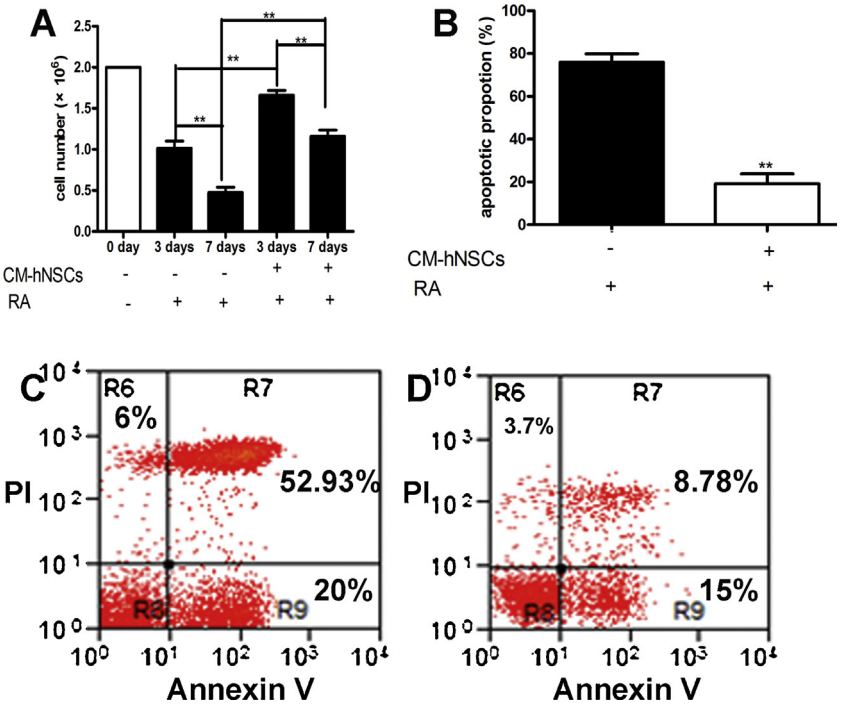

Fig. 1. CM-hNSCs significantly improved the survival of RA-treated SH-SY5Y cells.
(A) Bar graph showing that the cell number at the 3rd day was higher than that at the 7th day following RA treatment in the presence or absence of CM-hNSCs. However, note that the presence of CM-hNSCs significantly improved the cell survival rate, with a higher cell number than that in the absence of CM-hNSCs, at both of the 3rd and 7th day following RA treatment. One-way ANOVA, $^{**}P < 0.01$(B) Bar graph showing that the apoptotic percentage of RA-treated SH-SY5Y cells was significantly decreased in the presence of CM-hNSCs. Two-sample *t*-test, $^{**}P < 0.01$(C and D). The dot plots showing the representative flow cytometric results of PI/annexin V staining in the absence (C) and presence (D) of CM-hNSCs at the 7th day following differentiation of the RA-treated SH-SY5Y cells.