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Administration of BDNF/ginsenosides combination enhanced synaptic development in human neural stem cells

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ABSTRACT

Ginsenosides Rg1 and Rb1, major pharmacologically active ingredients from Ginseng, the root of Panax ginseng C.A. Meyer (Araliaceae), were applied in the differentiation media for human neural stem cells (hNSCs), together with brain-derived neurotrophic factor (BDNF), a commonly used compound for neural stem cell (NSC) differentiation. Cell locomotion and neurite extension were observed by time-lapse microscopy and analyzed by ImageJ software. The expression of synaptic formation was confirmed by immunostaining of synaptophysin (SYN) or/and the co-localization of synapsin I and microtubule associated protein-2 (MAP-2). Effects of cell density on neural differentiation were also examined. Results have shown that administration of BDNF/ginsenosides (Rg1 and Rb1) combination in differentiation medium promoted cell survival, enhanced neurite outgrowth and synaptic marker expression during differentiation. High cell density enhanced synaptic marker expression in BDNF/ginsenosides combination medium. In all, this study established a condition for hNSCs synaptic development in early differentiation, which is a crucial step in applying this cell line in neural network-based assay.

1. Introduction

Neural stem cells (NSCs), which are derived from embryonic stem cells (ESCs) are partially differentiated, multipotent and can develop into any type of neural cells (e.g. neurons and astrocytes) *in vitro* (Zhang, 2006). Also, NSCs are capable of proliferating, thus providing an unlimited cell source. In pharmaceutical and biotechnology industries, human NSCs (hNSCs) are being considered as a promising cell source in preclinical studies (Irion et al., 2008). While in tissue engineering and regenerative medicine, these cells are actively being investigated for neurodegenerative disease therapy (Ogawa et al., 2009). There is interest in differentiating hNSCs to mature functional neural networks for preclinical studies of drugs against connectivity related targets (e.g. N-methyl-D-aspartic acid receptors (NMDARs)) (Meador-Woodruff et al., 2003; Wang and Kisaalita, 2010; Wang et al., submitted for publication).

NSC differentiation is regulated by various microenvironment cues, such as chemical and physical properties of extracellular matrix (Little et al., 2008), and functional neural network development from NSCs has been proven to be microenvironment dependent (Illes et al., 2009). Among these effective molecules that affect neuronal fate (Schaffer and Gage, 2004), brain-derived neurotrophic factor (BDNF) is an effective modulator of hNSC differentiation and neuronal cell survival (Poo, 2001). It plays an important role in the survival of differentiated neural stem cells through the MAPK/ERK-dependent and PI3K/Akt-dependent Bcl-2 up-regulation (Lim et al., 2008). Also, it has been proven to accelerate the maturation of the synaptic vesicle protein synapsin-1 at developing neuromuscular junctions in cell cultures (Poo, 2001). Thus, BDNF has been often added in neural stem cell differentiation media (Johnson et al., 2007).

Ginseng, the root of Panax ginseng C.A. Meyer (Araliaceae), has been extensively used in traditional oriental medicine for over 2000 years. *In vivo* and *in vitro* studies have shown its beneficial effects in cardiovascular diseases, cancer and immune deficiency (Cheng et al., 2005). A recent study has suggested that some of ginseng's active ingredients also exert beneficial effects on aging and neurodegenerative diseases (Radad et al., 2006). Ginsenosides such as Rb1 (MW 1109.29 Da) and Rg1 (MW 801.02 Da) are the major pharmacologically active ingredients of ginseng and their anti-aging and anti-neurodegeneration effects have been well proven (Cheng et al., 2005). Cell-based studies have shown that ginsenosides promoted NSC proliferation *in vitro* and enhanced cell survival (Shen and Zhang, 2004). Mechanisms may involve decreasing NO content and NOS activity, reducing intracellular calcium concentration, by up-regulating Hes1 expression, enhancing superoxide dismutase (SOD) activity and enhancing the ratio of Bcl-2 to Bax protein and inhibiting activation of caspase-3 (Cheng et al., 2005; Zhuang et al., 2009). Ginsenosides Rg1 and Rb1 also promoted neurite outgrowth in PC12 cells (Rudakewich et al., 2001), enhanced astrocyte

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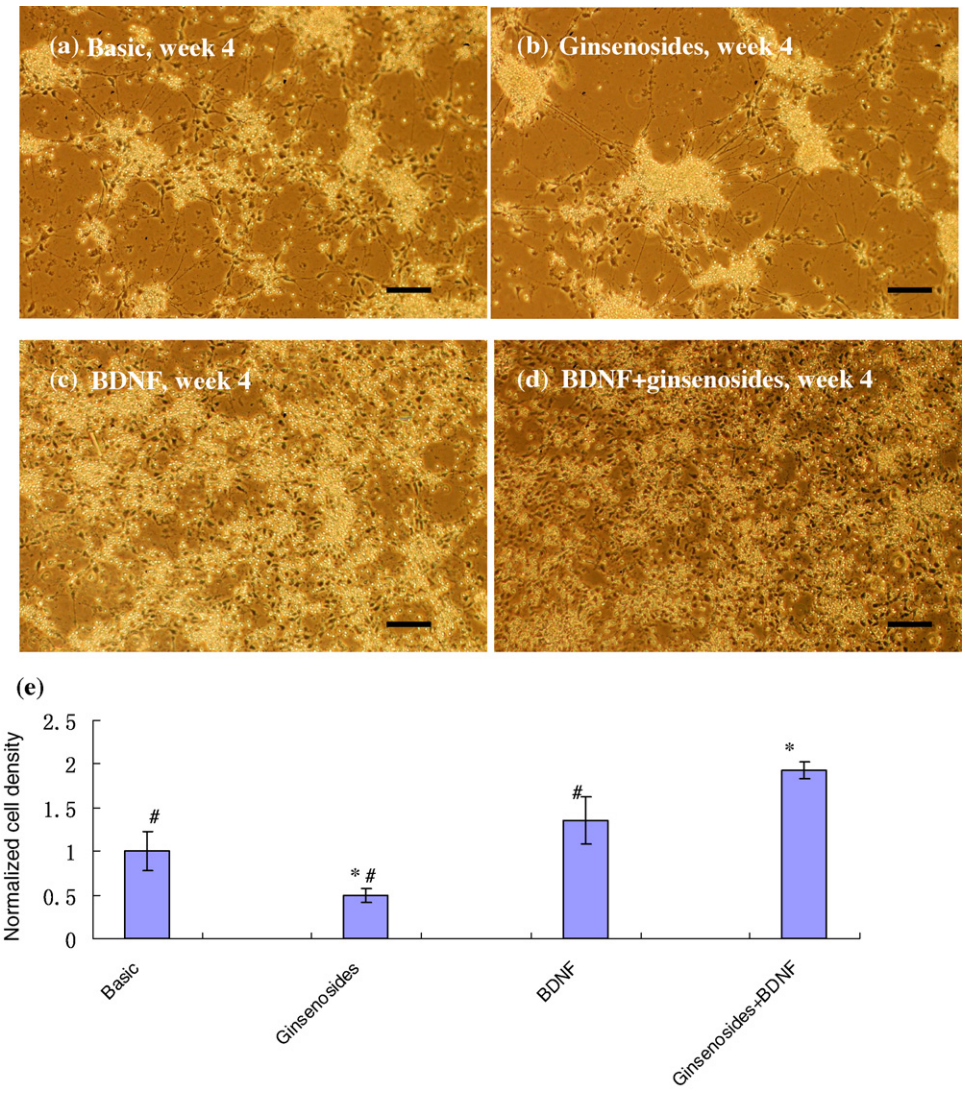

Fig. 1. (a–d) Phase contrast images of hNSCs at week 4 in differentiation in basic differentiation media (a), basic media with ginsenosides Rg1 (5 μM) and Rb1 (5 μM) (b), basic media with BDNF (10 ng/ml) (c), basic media with combination of BDNF (10 ng/ml) and ginsenosides Rg1 (5 μM) and Rb1 (5 μM) (d). Scale bar = 100 μm. (e) Cell densities (mean ± S.D.) were normalized by dividing with the mean density from the basic differentiation media group. $^{*}P < 0.05$, compared to the group with basic differentiation media; $^{\#}P < 0.05$, compared to the group with the combinational differentiation media.

differentiation from NSCs (Shi et al., 2005), increased neurotransmitter release (Xue et al., 2006), and increased synapse number and the density of synaptophysin, which is the morphological basis for explaining Rb1 and Rg1 induced facilitation of learning and memory (Mook-Jung et al., 2001).

In this study, we explored the effects of BDNF/ginsenosides (Rg1 and Rb1) combination on hNSC differentiation. Based on previous studies of BDNF and ginsenosides for neural cell survival and differentiation, it was expected that the combination could enhance synaptic development in hNSC differentiation than BDNF or ginsenosides, separately. We also studied the effects of cell density on hNSC differentiation with respect to synaptic formation. The goal was to establish a hNSC differentiation condition for neural network connectivity development, reflected by synaptic formation.

2. Materials and methods

2.1. Cell line and cell culture

hNSCs, purchased from Millipore (ENStem-A^{TM}, Billerica, MA), were derived from WA09 human ESCs using methods previously described (Shin et al., 2005). Cells were maintained as previously described (Wang and Kisaalita, 2010). Briefly, cells were cultured in 35 mm Petri dishes with 2 ml growth medium in a 5% CO_2 humidified atmosphere at 37 °C. ENStem-A^{TM} expansion media supplemented with penicillin/streptomycin, L-glutamine and basic fibroblast growth factor (bFGF) was used. The medium was half changed every other day. Cells were passaged by mechanically pipetting when cells reached 90–100% confluence and around 1.2×10^6 cells were seeded into each new dish. For cell differentiation, expansion media was replaced with differentiation media. Compared with expansion media, bFGF was withdrawn in basic differentiation media (Yan et al., 2005). Ginsenosides Rg1 (5 μM) and Rb1 (5 μM) (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China) and BDNF (10 ng/ml, Invitrogen, Carlsbad, CA) were added in basic differentiation media. The concentrations were decided based on previous studies (Johnson et al., 2007; Shi et al., 2005). There were four differentiation conditions: (1) basic differentiation media; (2) ginsenosides Rg1 (5 μM) and Rb1 (5 μM) in basic differentiation media; (3) BDNF (10 ng/ml) in basic differentiation media; (4) combination of BDNF (10 ng/ml) and ginsenosides Rg1 (5 μM) and Rb1 (5 μM) in basic differenti-