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The role of p38 MAP kinase and c-Jun N-terminal protein kinase signaling in the differentiation and apoptosis of immortalized neural stem cells

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Abstract

The two distinct members of the mitogen-activated protein (MAP) kinase family c-Jun N-terminal protein kinase (JNK) and p38 MAP kinase, play an important role in central nervous system (CNS) development and differentiation. However, their role and functions are not completely understood in CNS. To facilitate in vitro study, we have established an immortal stem cell line using SV40 from fetal rat embryonic day 17. In these cells, MAP kinase inhibitors (SP600125, SB202190, and PD98059) were treated for 1, 24, 48, and 72 h to examine the roles of protein kinases. Early inhibition of JNK did not alter phenotypic or morphological changes of immortalized cells, however overexpression of Bax and decrease of phosphorylated AKT was observed. The prolonged inhibition of JNK induced polyploidization of immortalized cells, and resulted in differentiation and inhibition of cell proliferation. Moreover, JNK and p38 MAP kinase but not ERK1/2 was activated, and p21, p53, and Bax were overexpressed by prolonged inhibition of JNK.

These results indicate that JNK and p38 MAP kinase could play dual roles on cell survival and apoptosis. Furthermore, this established cell line could facilitate study of the role of JNK and p38 MAP kinase on CNS development or differentiation/apoptosis.
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Keywords: Neural stem cells; c-Jun N-terminal protein kinase; p38 MAP kinase; Extracellular regulated kinase 1/2; Immortalization; Simian virus 40 (SV40) large

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1. Introduction

Neural stem cells (NSCs) are multipotential progenitor cells that are undifferentiated and capable of proliferation, self-renewal, and the production of many differentiated functional progenies [1]. Moreover, engraftment of these cells leads to recovery in neuropathological conditions [2,3]. NSCs possess self-renewal propensity in vitro growing in clonal aggregates called "neurospheres" and may provide an unlimited source of cells for grafting into patients with Parkinson's disease [4], Hungtington's disease [5], and multiple sclerosis [6]. For these reasons basic studies aiming to well characterize the biology of NSCs are of great interest. These cells are exposed to a high concentration of mitogens, such as epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF), and can be induced to differentiate by withdrawing the mitogens or by adding differentiating substances [7]. Behavior of NSCs in culture is affected by multiple variables: species, growth medium, and passage method (dissociated spheres, chopped spheres, and bioreactor) [8,9]. Moreover, the ability of immature progenitors and stem cells contained in the in vitro expanded spheres to differentiate in vivo into mature neurons and glia is still unclear. Recently, Milosevic et al. reported spontaneous apoptosis in murine free-floating neurosphere by activation of caspase-3 and Bcl-2 family [10].

The different members of the superfamily of mitogen-activated protein (MAP) kinases participate in signaling cascades conserved through evolution, which regulate important biological activities [11]. Three major groups of MAP kinases (MAPKs) exist: the p38 MAP kinase family, the extracellular signal-regulated kinase (Erk) family, and the c-Jun NH2-terminal kinase (JNK) kinase family [12–14]. Within the nervous system, MAPK pathways have been implicated in diverse physiological functions, including synaptic plasticity, gene expression and ion-channel activation [15]. In general, the ERK cascade is activated by growth factors and trasnduce signals to promote cell proliferation and survival [16,17]. Conversely, c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and p38 MAPK become activated by a variety of stress signals encompassing UV irradiation, inflammatory cytokines and oxidative stimuli and are implicated in the induction of apoptotic cell death [18]. However, controversial evidence has indicated that more complex roles of these MAPK pathways exist to transmit other ultimately distinct cellular effects in different cell lineages [19]. For example, it has been demonstrated that mitogen-activated protein kinase classes known as JNK, ERK, and p38 MAP kinase signals are generally involved in the neuronal differentiation [20,21].

Understanding in differentiation and apoptosis of NSC-derived cells demanded an in vitro model system for mechanistic approaches. However, the NSC-derived cells in the primary culture have a limited ability to differentiate and a rather short life span. In addition, difficulties of culture require large sacrifice of fetuses to obtain, and this has cost implications because of the large number of animals that would be required. These disadvantages have limited the application of primary cultures to wider investigations.

To investigate MAPK signaling pathway in differentiation and apoptosis of NSC-derived cells, this study was to use gene transfection techniques to establish an immortal cell line of murine NSC-derived cells. We used simian virus 40 large T (SV40) to integrate in immortalizing gene into the host genome. Using this approach, we were able to determine, in immortalized NSC-derived cells, the p38 and JNK signaling in the differentiation, and the early mediator of apoptosis.

2. Materials and methods

2.1. Isolation of primary neural stem cells from fetal rat brain

Neural stem cells were prepared by culturing cells from cerebrum of embryonic day 17 (ED17) Sprague–Dawley fetal rats (Bio Genomics, Seoul, Korea). Briefly, fresh cerebrums were dissociated in the presence of trypsin and DNase I (Sigma, St. Louis, MO, USA) and planted on petri dishes (Nunc, Nalge Nunc International Crop., IL, USA). Cells were seeded at a density of $(1–5) \times 10^4$ cells/ml in a mixture (1:1) of Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F12; GIBCO, Life Technologies, Grand Island, NY, USA) replenished with 2% B27 (GIBCO), gentamycin (50 μg/ml; GIBCO), anti-PPLO (pleuro-pneumonia-like organism) reagent (100 μg/ml; GIBCO), recombinant human basic

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