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Mechanisms of cell death of neural progenitor cells caused by trophic support deprivation

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Abstract

Cell death of neural progenitor cells is the primary problem limiting the value of neural progenitor cell-based therapy for central nervous system disorders. However, little is known about the mechanism of cell death of neural progenitor cells. In this study, we investigated the mechanisms of cell death of a multipotent cell line, MEB5, caused by deprivation of epidermal growth factor (EGF). When EGF was removed from the culture medium, the total number of viable MEB5 cells reduced, and nuclear condensation and elevation of caspase-3-like enzyme activity were observed in MEB5 cells. Treatment with a broad-range caspase inhibitor reduced cell death in a concentration-dependent manner, indicating that MEB5 cells undergo caspase-mediated apoptotic cell death caused by EGF deprivation. We also investigated the effects of glutamate receptor antagonists, antioxidants and nitric oxide synthase inhibitor on EGF deprivation-induced cell death. *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonists, α-amino-3-hydrozy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptor antagonist and nitric oxide synthase inhibitor failed to reduce cell death. In contrast, two antioxidants with different chemical structures reduced cell death in a concentration-dependent manner. The production of reactive oxygen species was detected in MEB5 cells after EGF deprivation by monitoring dichlorodihydrofluorescein fluorescence as a marker of reactive oxygen species-related radicals. Our results suggest that oxidative stress triggers caspase-mediated apoptosis of neural progenitor cells by trophic support deprivation.

Keywords: Neurogenesis; Neural progenitor cell; Trophic factor; Cell death; Antioxidant

1. Introduction

The development of therapeutic approaches for neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease has largely focused on modulating neurotransmitters in surviving neurons. These approaches have achieved some positive results; however, their therapeutic effects are not fully satisfactory. Recent studies have shown that neural stem cells and neural progenitor cells exist not only in the developing brain, but also in the subventricular zone and subgranular zone of the adult mammalian brain, including the human brain (Eriksson et al., 1988; Temple, 2001). They are capable of generating new neurons, astrocytes and oligodendrocytes. Using bromodeoxyuridine (BrdU) labeling, neurogenesis was found to decrease during aging due to the reduction of the proliferative activity of neural progenitor cells (Kuhn et al., 1996). The proliferative and survival activities of neural progenitor cells decreased in a transgenic mouse model of Alzheimer's disease, and amyloid β-peptide inhibited the proliferation and neuronal differentiation of cultured neural progenitor cells (Haughey et al., 2002). Moreover, transplantation of neural progenitor cells into the lateral ventricle of aged rats improved age-associated cognitive function (Qu et al., 2001), and activation of endogenous neural progenitor cells regenerated hippocampal pyramidal neurons and improved behavioral recovery after global ischemia of the brain (Nakatomi et al., 2002). These findings suggest new strategies for treating neurological or psychiatric disorders that are intractable under current therapies (Lie et al., 2004; Lindvall et al.,

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2004; Goldman, 2005). There are two approaches to achieve the potential utility of neural progenitor cells: transplantation of exogenous neural progenitor cells and mobilization of endogenous neural progenitor cells. It is hoped that neural progenitor cells will self-renew, migrate to lesioned brain regions, differentiate into neurons, integrate into the neuronal circuit and, finally, mediate functional recovery.

The development of neural progenitor cell-based therapy for central nervous system disorders is still at an early stage. In animal studies, less than 32% of human neural precursor cells survive after transplantation (Le Belle et al., 2004) and about 50% of newly generated cells in the adult brain survive (Petreanu and Alvarez-Buylla, 2002; Winner et al., 2002). Although the relationship between the number of surviving cells and functional recovery has not been established, it is quite likely that improving cell survival would have benefits for functional recovery. Therefore, one of the basic issues to be resolved is how to control the survival of neural progenitor cells. Several studies have been made to characterize the cell death of neural progenitor cells. In those studies, cell death was caused by extrinsic stimulants such as glutamate, hydrogen peroxide (H_2O_2) or rotenone (Hsieh et al., 2003; Lin et al., 2004; Brazel et al., 2005; Li et al., 2005). Moreover, it was reported that the engraftment efficiency of neural progenitor cells engineered to release glial cell line-derived neurotrophic factor (GDNF) in the adult mouse striatum was higher than that of the parental neural progenitor cells (Akerud et al., 2001). It is most likely that cell death of neural progenitor cells is caused by lack of trophic support, in addition to stimulant-induced cell death. However, the characteristics of cell death of neural progenitor cells caused by lack of trophic support remain unknown.

MEB5 cells are a multipotent mouse cell line established by Yoshida et al. (Nakagaito et al., 1998). MEB5 cells proliferate in response to epidermal growth factor (EGF). When EGF is withdrawn, about 50% of the cells die and the remaining cells begin to express the marker proteins for neurons, astrocytes or oligodendrocytes. The cells that develop neuronal morphology show a tetrodotoxin-sensitive inward sodium current like that observed in neurons. Thus, MEB5 cells provide a good cell system to investigate the molecular mechanisms of cell death of neural progenitor cells caused by trophic support deprivation. In this study, we characterized the proliferation, cell death and differentiation of MEB5 cells after EGF deprivation, and examined the effects of glutamate receptor antagonists, antioxidants and nitric oxide synthase inhibitor on the cell death of MEB5 cells caused by EGF deprivation.

2. Materials and methods

2.1. Materials

MEB5 cells (Registration No. IFO50472) were purchased from Health Science Research Resources Bank (Osaka, Japan). Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, amphotericin B and laminin were from Invitrogen Corp. (Carlsbad, CA, USA). EGF was from TAKARA BIO INC. (Kyoto, Japan). Transferrin was from Roche Applied Science (Penzberg, Germany). Insulin, biotin and fibronectin were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium selenite was from Nacalai Tesque (Kyoto, Japan). Poly-L-lysine, saponin, MK-801, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), N^{ω}-nitro-L-arginine methyl ester (L-NAME) and 7-amino-4-methylcoumarin (AMC)-DEVD were from Sigma (St. Louis, MO, USA). Normal goat serum was from Vector Laboratories Inc. (Burlingame, CA, USA). Anti-nestin monoclonal antibody and anti-microtubule associated protein-2 (MAP2) polyclonal antibody were from CHEMICON International Inc. (Temecula, CA, USA). Anti-glial fibrillary acidic protein (GFAP) polyclonal antibody was from DakoCytomation (Glostrup, Denmark). Cy2-conjugated goat anti-mouse IgG and Cy3-conjugated goat anti-rabbit IgG were from Jackson ImmunoResearch LABORATORIES, INC. (Pennsylvania, USA). 4′,6′-Diamidino-2-phenylindole (DAPI) and 5,6-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetete (CM-H_2DCFDA) were from Molecular Probes (Eugene, OR, USA). FluorSaveTM Reagent, EUK-8 and Mn(III)tetrakis(4-benzoic acid)porphyrin (MnTBAP) were from CALBIOCHEM (San Diego, CA, USA). Z-VAD-FMK was from Promega Corp. (Madison, WI, USA). The protein assay kit was from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). The Cytotoxicity Detection LDH kit was from Kyokuto Pharmaceutical Industrial Corp. (Tokyo, Japan). All other reagents were purchased from Sigma or Wako Pure Chemical Industries, Ltd.

2.2. Cell culture

MEB5 cells, a multipotent cell line, were propagated as floating neurospheres in DMEM supplemented with 5 μg/ml insulin, 10 ng/ml EGF, 50 μg/ml transferrin, 10 ng/ml biotin, 30 nM sodium selenite, 100 U/ml penicillin, 100 μg/ml streptomycin and 250 ng/ml amphotericin B (MEB5 standard culture medium). Cultures were incubated at 37 °C in a 95% air/5% CO_2 humidified atmosphere. Floating neurospheres were dissociated and plated on 6-well plates, 24-well plates, 96-well plates or 8-well chamber slides coated with poly-L-lysine, laminin and fibronectin in MEB5 standard culture medium at a density of 1.0×10^5 cells/cm^2. Medium was replenished every 2 d. For the measurement of caspase activity, cells were plated on poly-L-lysine/laminin/fibronectin-coated 6-well plates at 1.5×10^5 cells/cm^2.

2.3. Imunocytochemistry

MEB5 cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min and washed with PBS. The fixed cells were incubated for 30 min with a solution containing 5% normal goat serum and 0.005% saponin to block nonspecific antibody binding. The cells were immunostained for a marker of progenitors (nestin), neurons (MAP2) or astrocytes (GFAP). Antibodies against nestin, MAP2 and GFAP were diluted in a PBS solution containing 1% normal goat serum and 0.005% saponin at dilutions of 1:500, 1:1000 and 1:800, respectively. The cells were incubated overnight at 4 °C with a primary antibody, washed with PBS, and incubated 1.5 h