Available online at www.sciencedirect.com

SCIENCE DIRECT

Brain Research Protocols 15 (2005) 52 – 58

BRAIN RESEARCH PROTOCOLS

www.elsevier.com/locate/brainresprot

Protocol

A modified method for generation of neural precursor cells from cultured mouse embryonic stem cells

Haiwei Xu[a,1], Xiaotang Fan[b,*,1], Jun Tang[a], Guangji Zhou[a], Li Yang[a], Xuan Wu[a], Shingyong Liu[c,2], Jifu Qu[d,2], Hui Yang[c,*]

[a]*Department of Physiology, The Third Military Medical University, Chongqing 400038, P.R. China*
[b]*Department of Neurobiology, The Third Military Medical University, Chongqing 400038, P.R. China*
[c]*Department of Neurosurgery, Xinqiao Hospital, The Third Military Medical University, Chongqing 400038, P.R. China*
[d]*Department of Emergency, Southwest Hospital, The Third Military Medical University, Chongqing 400038, P.R. China*

Accepted 23 March 2005
Available online 25 April 2005

Abstract

The pluripotency and high proliferative capacity of embryonic stem (ES) cells make them an attractive source of different cell types for biomedical research and cell replacement therapies. It has been demonstrated that ES cells can be induced into neural precursor cells (NPCs) under conditions. NPCs can be expanded in large numbers for significant periods of time to provide a reliable source of cells for transplantation in neurodegenerative disorders and injury of the central nervous system. This study describes a modified method for generation of NPCs from cultured mouse ES cells.

Theme: Development and regeneration
Topic: Cell differentiation and migration

Keywords: Embryonic stem cells; Neural precursor cells; Cell culture

1. Introduction

The ability to isolate, proliferate, and genetically manipulate embryonic stem (ES) cells is one of the major achievements in experimental biology [2,9]. ES cells are pluripotent cell lines which provide a source of cells for tissue engineering applications because of their ability to differentiate to all somatic cells and their unlimited proliferative capability. The controlled differentiation of ES cells into stem cells of defined lineages may eventually lead to alternative donor sources for tissue reconstruction [1]. ES cells would be candidates for cell replacement therapies for degenerative diseases, but direct transplantation of ES cells may result in teratoma [16,20], while neurons and glial cells are post-mitotic cells and lost the ability of proliferation when grafted to the brain, and neurons cannot differentiate according to the environmental cues or migrate to the injury areas of the brain. Thus, the neural precursor cells (NPCs) with the properties of neural stem cells differentiated from ES cells would be suitable candidates for cell replacement [15]. However, to use ES cells in cell replacement therapies for degenerative diseases, a number of challenges must be addressed regarding methods to culture and direct ES cell differentiation to

* Corresponding authors. Xiaotang Fan is to be contacted at Department of Neurobiology, The Third Military Medical University, Chongqing 400038, P.R. China. Fax: +86 23 68752253. Hui Yang, Department of Neurosurgery, Xinqiao Hospital, The Third Military Medical University, Chongqing 400038, PR China.

E-mail addresses: fanxiaotang@163.net (X. Fan), yanghxqh@cta.cq.cn (H. Yang).

[1] These authors contributed equally to this work.

[2] These authors helped to revise the paper and did the quantitation of the results.

1385-299X/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainresprot.2005.03.004

NPCs. For example, the reported percentile of cells expressing neuronal markers is usually low and does not exceed 30% [11]. Generation of specific neuronal populations in sufficient quantities for transplantation represents a significant practical problem. This protocol presents a detailed and modified method of mouse ES cell culture and its differentiation into NPCs. The author's own experiences were also described in this protocol.

2. Type of research

Culture of embryonic stem cells and differentiation into neural precursor cells.

Expansion and differentiation of neural precursor cells.

3. Time required

Step 1 Mouse embryonic fibroblast (MEFs) preparation: 15–17 days
Step 2 ES cell culture and embryoid body (EBs) formation: 7 days
Step 3 Alkaline phosphatase activity detection of ES cells: 2 days
Step 4 Selection of NPCs: 5–7 days
Step 5 Expansion of NPCs: up to 6 days
Step 6 NPCs detection: 2 days
Step 7 Differentiation of NPCs: 14 days

Total: 51–55 days, depending on the MEF passages used as feeder and the expansion of NPCs.

4. Materials

For the culture of mouse ES cells and differentiation to NPCs, the following materials are needed. All solutions, surgical equipment, and tissue culture plates should be sterile.

4.1. Animals

MEFs were isolated from 13.5-day mouse embryos provided by Animal Breeding Center of The Third Military Medical University (Chongqing, China). Successful mating was confirmed by the presence of sperm in vaginal smears the next morning, and the resultant embryos were termed E0 according to Paxinos et al. [13].

4.1.1. Special equipment

Surgical equipment including two pairs of fine-tipped watchmaker's forceps and small surgical scissors, 15 ml plastic centrifuge tubes (Eppendorf), Pasteur pipettes (Falcon), culture dish (Falcon), membrane filter unit (0.22 μm) (Millipore), dissecting microscope (Olympus), Benchtop centrifuge (Clements), humidified CO_2 incubator (Heraeus), circular coverslips, 13-mm diameter, no. 1 thickness (BDH), 70-mm bacteriological dishes (Greiner), compound microscope (Zeiss), inverted compound microscope with phase contrast optics (Olympus), and spot insight COOL CCD Camera (DIAGNOSTIC Instrument Inc).

4.1.2. Chemicals and reagents

Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, high glucose), Dulbecco's modified Eagle's medium/F12 (DMEM/F12, GIBCO), fetal bovine serum (FBS; Hyclone), Mitomycin-C (Sigma,10 mg/l), human leukemia inhibitory factor (hLIF, Chemicon), beta-mercaptoethanol (Sigma,100 μM, diluted with PBS), trypsin solution (0.05% trypsin and 0.05% EDTA; Life Technologies), poly-L-ornithine (Sigma,15 μg/ml, diluted with PBS), fibronectin (Sigma, 10 μg/ml, diluted with DMEM/F12), laminin (GIBCO, 5 μg/ml, diluted with DMEM/F12), N2 supplement (GIBCO, 50 ×, contains insulin 500.00 μg/ml, human Transferrin 10,000.00 μg/ml, progesterone 0.63 μg/ml, putrascine 1611.00 μg/ml, and selenite 0.52 μg/ml, diluted with DMEM/F12), basic fibroblast growth factor (bFGF, Sigma, 20 ng/ml, diluted with DMEM/F12), epidermal growth factor (EGF, human recombinant, Sigma, 20 ng/ml, diluted with DMEM/F12), B27 (GIBCO, 2% diluted with DMEM/F12), normal goat serum (Sigma), 3,3′-diaminobenzidine (Sigma), monoclonal anti-nestin antibody (1:1500, Pharmergen), monoclonal anti-O4 antibody (1:2000, Chemicon), monoclonal antibody against NF-200 (DAKO), polyclonal antiserum against glial fibrillary acidic protein (GFAP, DAKO), ABC kit (Vector Laboratories), secondary antibodies: anti-rabbit biotin and anti-mouse-biotin (DAKO).

4.2. Solutions

4.2.1. Water

Water was purified by osmosis and through a Milli-QUF plus water purification system (Millipore), and was autoclaved.

4.2.2. Phosphate-buffered saline (PBS)

To prepare 1 l, dissolve in water 0.2 g KCl, 0.195 g KH_2PO_4, 8.12 g NaCl, and 0.97 g Na_2HPO_4, and adjust pH to 7.2 with HCl. PBS was sterilized by filtration and stored at 4 °C.

4.2.3. Dulbecco's modified Eagle's medium (DMEM, high glucose)

To prepare 1 l, dissolve 10 g in 1 l water, add 2.0 g of $NaHCO_3$ and 2.0 g HEPES, adjust pH to 7.0 with 1 N HEPES, then sterilize DMEM by filtration, and aliquots stored at −20 °C for up to 3 weeks.

4.2.4. Fetal bovine serum (FBS)

FBS was thawed at 37 °C and aliquots stored at −20 °C.