Journal of College of Medical Sciences-Nepal, 2014, Vol-10, No-2 *Original Article*

Neural stem cell isolation and culture from C57BL/6 mice

Koirala S[1], Shah S[1], Khanal L[2]

[1] Assistant Professor, [2]Post graduate resident, Department of Human Anatomy, BP Koirala Institute of Health Sciences, Dharan, Nepal

ABSTRACT

INTRODUCTION

A widely used in vitro culture, the neurosphere assay (NSA) has provided a means to retrospectively identify neural progenitor cells as well as to determine both their self-renewal capacity. Objective of study was to isolate and compare growth of the embryonic neuronal stem cell and adult neuronal stem cells in presence of Epidermal Growth Factor (EGF) and Fibroblastic Growth Factor (FGF2).

MATERIALS AND METHODS

Embryonic neuronal stem cell were collected from cortical plate of dorsal telencephalon of fifteen C57BL/6 transgenic mice using stereoscopic microscope on 11th gestational day (GD). Adult mammalian neuronal stem cells taken from subventricular zone (SVZ) of the lateral ventricles and subgranular layer of the dentate gyrus of the hippocampus were cultured. The growth for the neurosphere was then observed in interval of 24 and 72 hours.

RESULT

The adult stem cell culture showed few intact cells with high amount of debris and 9% heterogeneous sphere after 24 hours while only 20 % was observed at the end of 72 hours. Higher proliferation rate was observed in embryonic neurospheres than the adult stem cell culture.

CONCLUSION

Presence of EGF and basic FGF2 is essential for culture of neurospheres.

Keywords: In vitro culture, neurosphere assay, stem cell

INTRODUCTION

The ability to prospectively identify and characterize neural progenitor cells in vivo has been difficult due to a lack of cell-surface markers. A widely used in vitro culture method known as the neurosphere assay has provided a means to retrospectively identify neural progenitor cells as well as to determine both their self-renewal capacity and their ability to generate neurons, astrocytes, and oligodendrocytes.[1] Combined with the establishment of multiple transgenic mouse strains expressing fluorescent markers and advances in cell isolation techniques, Neurosphere Assay provides a powerful system to prospectively elucidate neural progenitor characteristics and functions

Correspondence: Dr. Sarun Koirala
Email: poksun@rediffmail.com

The objective of this study was to isolate**,** culture and compare growth of neural stem cell from the embryonic cells, SVZ of the lateral ventricles and sub-granular layer of dentate gyrus of hippocampus of C57BL/6 mice in presence of EGF and FGF2.

MATERIALS AND METHODS

Embryonic neuronal stem cell**s** were collected from cortical plate of dorsal telencephalon of fifteen C57BL/6 transgenic mice using stereoscopic microscope on 11th GD. Adult mammalian neuronal stem cells were taken from SVZ of the lateral ventricles and subgranular layer of the dentate gyrus of the hippocampus then followed by chopping, trypsinization and cultured in T- 25flask with the plate coated with poly-l-ornithine in Dulbecco's minimum essential medium (DMEM) at 37^{0}C,5 % with CO_2 for 5 days in presence of mitogens EGF and FGF2. The growth for the neurosphere was then observed in interval of 24 and 72 hours. The study was carried as a part of neuroscience school.

RESULTS

After 24 hours: Generation of clonally derived heterogeneous sphere, clusters of undifferentiated cells termed Neurospheres of bigger size were present in culture taken from embyronic stem cell and in SVZ and sub-granular layer of dentate gyrus of hippocampus . The adult stem cell culture showed few intact cells with high amount of debris and 9% heterogeneous sphere.

At the end of 72 hours: Higher proliferation rate (40%) was observed in embryonic neurospheres than the adult stem cell culture in the presence of EGF and FGF2 (fig. 3,4). The adult cell culture showed neurospheres with less in number 20% .

DISCUSSION

Cell culture system designed to isolate and propagate putative stem cells from neural tissue has enhanced our ability to regulate their capacity to self-renew and to differentiate into neurons, astrocytes and oligodendrocytes.[2,3]In the presence of EGF and FGF these cell populations can be reliably expanded and maintained in the form of neurospheres.[4,5] Our study shows Neurospheres in embryonic culture showed a high rate of cellular proliferation in comparison to adult SVZ cells in presence of FGF2.Neurosphere culture formation is highly sensitive procedure. Variation in cell density, different constituents or concentrations of factors in the media and method creates a problem in consolidating and interpreting data even within the same study.[6] Only a small percentage of cells within each heterogeneous sphere have the potential to form neurospheres and even fewer cells actually fulfill the criteria for being neural stem cells.

CONCLUSION

Embryonic and adult neuronal cell culture was successfully established. Presence of EGF and basic FGF2 is essential for culture of neurospheres. Generated neurospheres can also be differentiated in the absence of mitogens, either as whole neurospheres to demonstrate their multipotency through their production of neurons, astrocytes and oligodendrocytes.

REFERENCES

1. Bez A, Corsini E, Curti D, et al. Neurosphere and neurosphere-forming cells: morphological and ultrastructural characterization. Brain Res. 2003; 993: 18-29.

2. Reynolds BA**,** Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science, 1992; 255: 1707-10.

3. Gerd K. Adult Neurogenesis. Oxford University Press, 2006; 66-78.

4. Kawaguchi A, Miyata T. Nestin-EGFP Transgenic Mice: Visualization of the Self-Renewal and Multipotency of CNS Stem Cells Molecular and Cellular Neuroscience. 2001;17: 259–73.

5. Vescovi AL, Reynolds BA, Fraser DD**,** et al**.** bFGF regulates the proliferative fate of unipotent (neuronal) and bipotent (neuronal/astroglial) EGF-generated CNS progenitor cells. Neuron, 1993; 11: 951-66**.**

6. Josephine BJ**,** Parmar M**.** Strengths and Limitations of the Neurosphere Culture System. Molecular Neurobiology 2006; 34:152-61**.**