ENStem-A Adherent Human Neural Progenitors: A New Source of Primary Human Neural Cells

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Abstract

Progenitor cell populations derived from human embryonic stem cells (hESCs) can serve as a renewable source of physiologically relevant human primary neural cells for basic research and drug discovery. Repeated derivation of neural progenitors from hESCs can lead to increased variability, along with the difficulty and expense of hESC culture. Millipore's new ENStem-A cells offer an attractive alternative: they proliferate as an adherent monolayer in serum-free, feeder-free conditions. Here we discuss the characteristics and functional assays in which ENStem-A cells can be used.

Introduction

Most neurons are produced during embryonic development by neural stem cells or neural progenitor cells. A new method to derive neural progenitors from NIH-approved human embryonic stem cells (hESCs)[1], has been licensed by Aruna Biomedical Inc. from the University of Georgia. This novel method allowed the development of a convenient, ready-to-use kit containing cryopreserved neural progenitors and the reagents required to propagate and differentiate them.

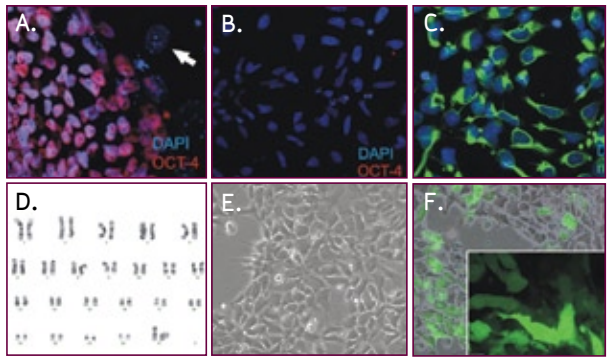

Figure 1. ENStem-A cells demonstrate the expected immunoreactivity, chromosome number, and transduction capacity. (A) WA09 human ESC are Oct-4 positive, while mouse feeder cells are negative (arrows). (B) ENStem-A cells are Oct-4-negative. (C) ENStem-A cells labeled for nestin immunoreactivity and display normal karyotype (D) and genetics consistent with their WA09 origin. (E) Phase contrast image of progenitors growing in an adherent monolayer. (F) Cells can easily be transduced with reporter genes demonstrating cells that are expressing GFP after lenti-viral transduction.

The cells and kit are produced by Aruna Biomedical and distributed by Millipore under the ENStem-A brand name.

Aside from their convenience, ENStem-A cells possess many useful properties. They are not transformed, making them particularly interesting for studying cell cycle regulation and proliferation because pathways involving histone acetyltransferases have not been altered or over expressed. Additionally, since the cells were derived from a registered stem cell line (WA09), the genetic background of the original donor is well documented. These cells have a normal compliment of chromosomes (euploidy) and are preserved without any genetic modifications to alter endogenous signaling pathways (Figure 1). Due to their proliferative capacity, they are amenable to genetic manipulations if this is desired by the end-user (Figure 1). ENStem-A cells have been shown to differentiate into multiple neural phenotypes including glia and motoneurons[1,2]. Use of growth factors and/or genetic markers will no doubt be useful for enrichment of neural cell phenotypes. They also do not express high levels of pluripotent markers like Oct-4 and are nestin positive.

Methods and Results

DRUG DISCOVERY

Though we have routinely grown ENStem-A cells in a variety of formats ranging from 35 mm dishes to T-300 flasks on poly-ornithine and laminin coated plates, we have recently demonstrated that they can also be grown in 96-well plates. Substrates tested for this format include collagen IV, fibronectin, and vitronectin, offering greater assay versatility (Figure 2). Differentiating these cells using our optimized ENStem-A differentiation media results in β-III tubulin positive cells which can be plated into 96-well plates and assayed. We have demonstrated the presence of functional AMPA receptors on these differentiated populations by using a Flex Station Fluorometer (Molecular Devices) to record dose-dependent changes in internal calcium concentration in response to AMPA (Figure 3).

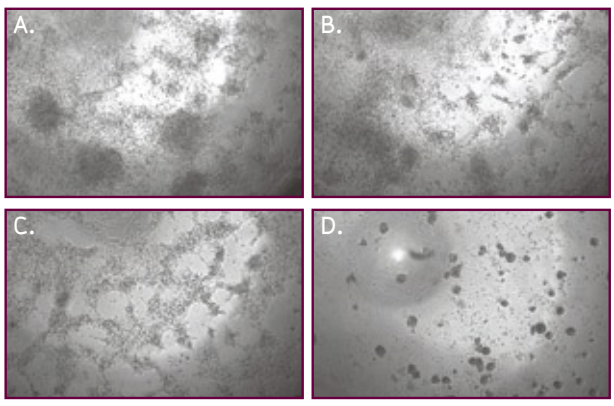

Figure 2. ENStem-A cells can be grown in 96 well plates on a variety of substrates. Phase contrast images at using 4x objective growing ENStem-A cells on collagen IV (A), fibronectin (B) and vitronectin (C). Cells plated without substrate on BSA treated wells did not grow plate efficiently.

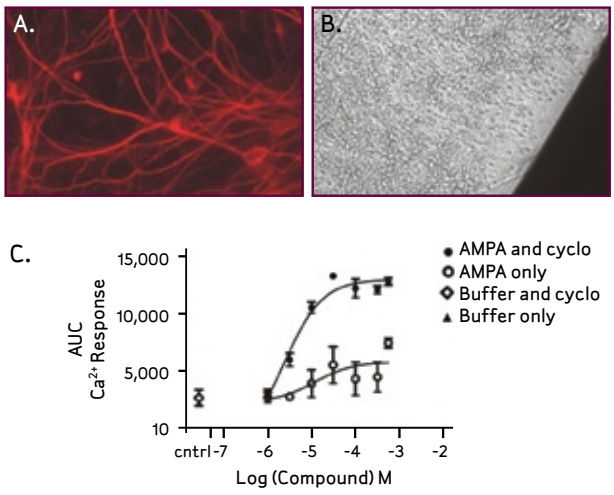

Figure 3. Differentiation and pharmacology. (A) ENStem-A cells differentiated for greater than 2 weeks are highly β-III positive. These differentiated cultures can be plated in 96 well plates (B) and assayed for changes in internal calcium concentration following agoinst application (C). Here we demonstrate that application of 50 μM cyclothiazide results in a greater influx of calcium in response to increasing doses of AMPA.

GENETIC MODIFICATIONS

Our recently published work on developing a drug screening assay for spinal muscular atrophy demonstrates that reporter genes can be introduced and expressed in ENStem-A cells[3]. This preliminary work may lead to new assays for other neuronal diseases and developmental neurotoxicity. Genetic reporters also help elucidate cellular function and track molecular pathways involved in development and differentiation.

We have also tested several means of transfecting and transducing ENStem-A cells. All lines were maintained on poly-ornithine laminin per our optimized protocols. To understand how efficiently these cells can be genetically manipulated, we used three very different systems (eletroporation by Nucleofector$_{[MC1]}$, transfection by ExGen500$_{[MC2]}$ and transduction by a lentiviral system) to deliver a green fluorescent protein (GFP) sequence under the control of the murine ubiquitous promoter, Ubiquitin C. To quantify the efficiency of delivery, we measured GFP expression by flow cytometry after 72 hours. At this time the number of live and dead cells were determined using propidium iodide. Transduced cells were further propagated to determine expression levels over time.

Based on percent GFP+ live cells, transduction by the lentiviral system was the most efficient. 35% of transfected ENStem-A cells expressed GFP+ and could be further purified using FAC >90% (Figure 4); transfection with ExGen500 (MBI Fermentas) was the least efficient with less than 3% GFP+ cells, and nucleofection gave mid-range results of 16% GFP+ cells. We also evaluated cell death with these three delivery techniques. The ENStem-A cells tolerated lentiviral transduction (~40% survival) more readily than the other techniques which had survival rates of 6% and 13% for nucleofection and ExGen 500, respectively. We continued culturing transduced ENStem-A cells for >15 passages to show that they retained their differentiation potential. ENStem-A cells can be modified using various techniques; however, when considering three parameters (% initial GFP+, viability and long term GFP expression), the lentiviral method performed better than other gene insertion methods tested.

Conclusion

ENStem-A cell monolayer cultures are uniform, robust, easily propagated, and capable of differentiating into many neural lineages. They grow on multiple substrates, can be genetically modified, and develop functional receptors which can be screened in a 96 well format. Thus, these cells are useful for many assays including neuroprotection, neurite outgrowth, synaptogenesis, growth cone collapse, genotoxicity, cell cycle regulation, DNA repair, cellular pathways (phenotypic differentiation, intracellular signaling), and pharmacology (dose response relationships, receptor desensitization).

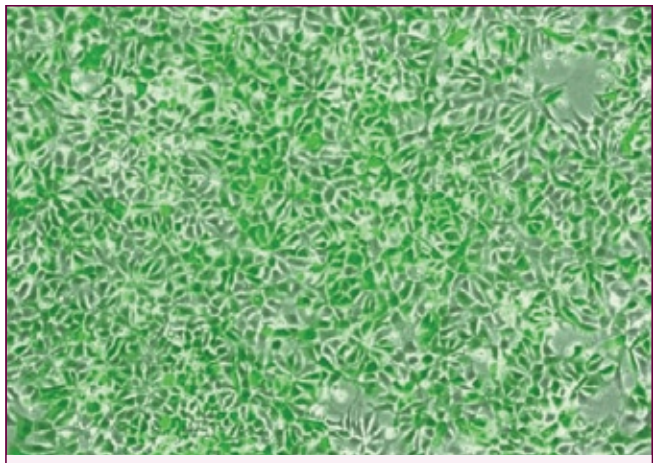

Figure 4. Lentiviral transduction of constitutive GFP expression. A phase-contrast superimposition onto GFP fluorescence demonstrates that roughly 90% of cells are transduced in initial experiments. Image courtesy of Aruna Biomedical.

Millipore Products

Description	Cat. No.
ENStem-A Human Neural Progenitor Expansion Kit	SCR055
ENStem-A Neural Freezing Medium, 50 mL	SCM011
ENStem-A Neural Expansion Medium, 500 mL	SCM004
ENStem-A Neuronal Differentiation Medium, 100 mL	SCM017

References

1. Shin, S. Mitalipova, M., Noggle, S., Tibbitts, D., Venable, A., Rao, R.R. Stice, S.L. (2006). *Stem Cells.* **24**: 125-38.
2. Shin, S., Dalton, S. Stice, S.L. (2005). *Stem Cells and Dev.* **14(3)** 266-9.
3. Wilson, PG, Cherry, J, Schwamberger, S, Adams, A, Zhou, J., Shin, S, and S. L. Stice. (2008). *Stem Cells and Development.* **Dec;16 (6)**:1027-42.

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