

Abstract:

DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELL DERIVED NEURAL PROGENITORS INTO FUNCTIONALLY RESPONSIVE POPULATIONS IN THE ABSENCE OF EXOGENOUS EGF

D.W. Machacek, S. K. Dhara, C. Sturkie, K. Hasneen, D. Carter, L. Murrah Hanson, P.R. MacLeish, M. Benveniste, S.L. Stice

The human nervous system originates from neuroepithelial cells. We have isolated, and maintained in culture, neural progenitors demonstrating properties of these early neural epithelial cells from WA09 human embryonic stem cells (hESCs). In the rodent it has been established that separate lineage-related epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) responsive neural stem cells exist. Most investigators maintaining hESC- derived human neural progenitors report growing in both EGF and FGF. Understanding the most basal culturing conditions under which these cells can form functional neural cultures is critical for allowing us to determine the signaling mechanisms by which these cells develop, and for demonstrating the robustness of hESC- derived tissues for cell therapy strategies where delivering exogenous support would be a further complicating variable.

Here we demonstrate that hESC- derived neural progenitors (ENStem-A, Millipore) do not require exogenous EGF for either long-term maintenance of the proliferative progenitor population nor the long-term survival of differentiated cultures. Human neural progenitors were maintained with bFGF and leukemia inhibiting factor (LIF) and differentiated by the withdrawal of bFGF for more than 17 weeks in the absence of EGF and other neurotrophic support. RNA was isolated at 32 and 125 days following removal of bFGF and gene expression was monitored using real time qRT-PCR. Importantly, we demonstrate that despite these basal conditions, we have an up-regulation of a variety of genes consistent with a diverse range of phenotypes including astrocytes and GABAergic, cholinergic, glutamatergic, dopaminergic, serotonergic and PNS neurons (GAD, GFAP, VACHT, EAAT, DAT, SERT, PRPH). Additionally we demonstrate the regulation of several genes suggesting functional maturation (KCC2, NAV 1.2, synaptophysin). Following two weeks of differentiation the expression of multiple glutamate receptor subunits was detected and functional responses to AMPA and kainic acid could be demonstrated in the presence of cyclothiazide using calcium imaging (FLIPR assay). Electrophysiological recordings 20-25 days following withdrawal of bFGF demonstrated cells with TTX sensitive inward sodium currents in the range of 800 pA and action potentials were recorded. Although the ENStem-A cells can be differentiated in serum free conditions, addition of 5% serum leads to earlier detectable levels of GFAP and increased expression of the genes PRPH, TH but decreased expression of VACHT suggesting that the fate of these cells is flexible in response to exogenous signaling cues.

This is the first demonstration that hESC derived neural stem cells can form long-term cultures of functionally responsive neural populations under such basal media conditions. In summary, hESC derived neural stem cells are robust enough to support themselves and create phenotypically diverse and functionally responsive neural cultures in the absence of exogenous EGF.