J. Cell. Mol. Med. Vol 15, No 6, 2011 pp. 1429-1431

Cortical network from human embryonic stem cells

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Received: September 15, 2010; Accepted: March 1, 2011

Abstract

The connection of embryonic stem cell technology and developmental biology provides valuable tools to decipher the mechanisms underlying human brain development and diseases, especially among neuronal populations, that are not readily available in primary cultures. It is obviously the case of neurons forming the human cerebral cortex. In the images that are presented, the neurons were generated *in vitro* from human embryonic stem cells *via* forebrain-like progenitors. Maintained in culture for prolonged time, they acquired a mainly glutamatergic phenotype and morphological characteristics of cortical pyramidal neurons, including dendritic spines, and formed spectacular networks.

Keywords: human embryonic stem cells • corticogenesis • neuronal culture • pyramidal projection neurons • dendritic spines

Beyond its implications in the understanding of human cortex development, the generation of human cortical neurons *in vitro* opens new venues for modelling diseases in which specific neuronal populations are affected. Recent methods directed the differentiation of mouse embryonic stem (mES) cells towards cortical neurons, following the development principles [1–4].

For the differentiation of human embryonic stem (hES) cells towards forebrain neural progenitors and cortical neurons, several development-related steps were also performed [1, 5–7].

The images that are presented illustrate neural progenitors and neurons derived from the hES cell line 401 (kindly provided by Prof. Outi Hovatta, Karolinska Institute, Stockholm, Sweden; ethical approval for culturing and *in vitro* differentiation of hES cells was obtained by the 'Carol Davila' University of Medicine and Pharmacy, Bucharest, Romania). Immunocytochemistry was performed with the following primary antibodies: forkhead box G1 (FOXG1) (rabbit IgG; Santa Cruz Biotechnologies, Santa Cruz, CA, USA), Forse1 (mouse IgG; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA, USA), tau (mouse IgG, Santa Cruz or rabbit IgG; Dako, Glostrup, Denmark) and glutamate (rabbit IgG; Sigma-Aldrich, St. Louis, MO, USA) and 488/555 Alexa-conjugated secondary antibodies. Images were taken with an ApoTome Imaging System based on Axio Observer.Z1 (Carl Zeiss Microscopy, Jena, Germany) using AxioVision software.

First, neuroepithelial cells were induced from hES cells, in chemical-defined cell culture conditions, without morphogens, and they acquired an anterior phenotype [5–7]. They differentiated into neural progenitor cells expressing the forebrain-related transcription factor FOXG1 (Fig. 1A) and the forebrain membranar marker Forse1 (Fig. 1B), and started to generate neurons (Fig. 1A, B).

The hES cell derived neurons were maintained in culture for prolonged time, with optimized culture conditions, and formed spectacular networks (Fig. 2A), where the majority acquired a glutamatergic phenotype (Fig. 2B). They presented pyramidal like-morphologies (Fig. 3A) and, like a novel aspect of hES cell derived neuronal morphology, dendritic spines with specific cortical morphologies could be identified *in vitro* (Fig. 3A, B), as was recently shown for the mES cell derived cortical neurons [8]. The subtype-related cortical neuronal specification and the functional characterization *in vitro* are under investigation.

Although cortical neuron replacement therapy appears as a relatively distant prospect, it was recently demonstrated that mES cell derived cortical progenitors have the remarkable ability to engraft and connect in the adult brain [8]. Another exciting and more immediate possibility will be to use the directed cortical differentiation systems to induced pluripotent stem cells [9] derived from patients presenting cortical diseases. Taking into consideration that the existing animal models are partially inadequate, this approach would provide an opportunity to model human cortical diseases.

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doi:10.1111/j.1582-4934.2011.01309.x

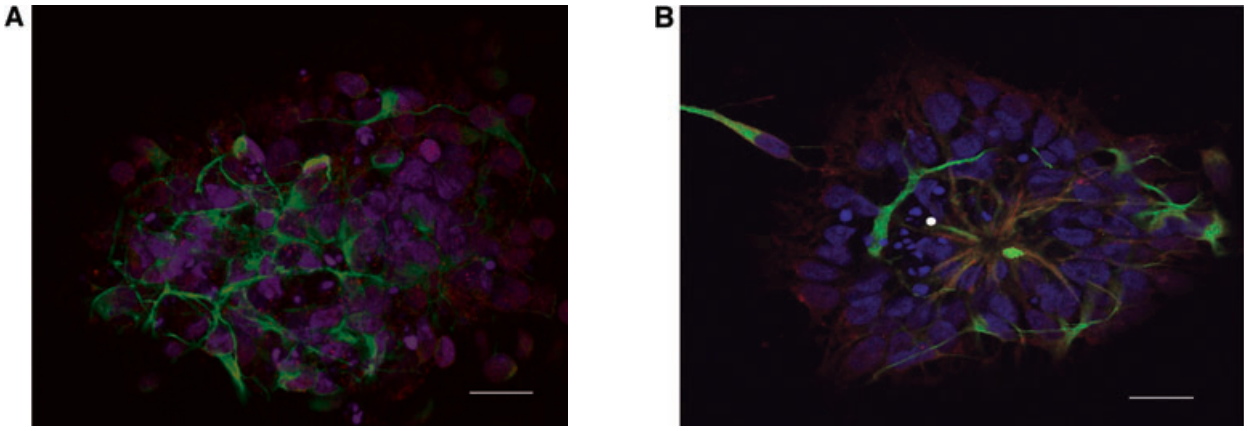

Fig. 1 Generation of neurons from hES cell derived forebrain-like progenitors. Neural progenitors and neurons derived from hES cells after 22 days of differentiation *in vitro*, expressing the forebrain-related transcription factor FOXG1 (red) (**A**) and the forebrain-related membranar marker Forse1 (red) (**B**). Green, tau-stained neurons and blue, Hoechst 33342 stained nuclei. Scale bar: 20 μm.

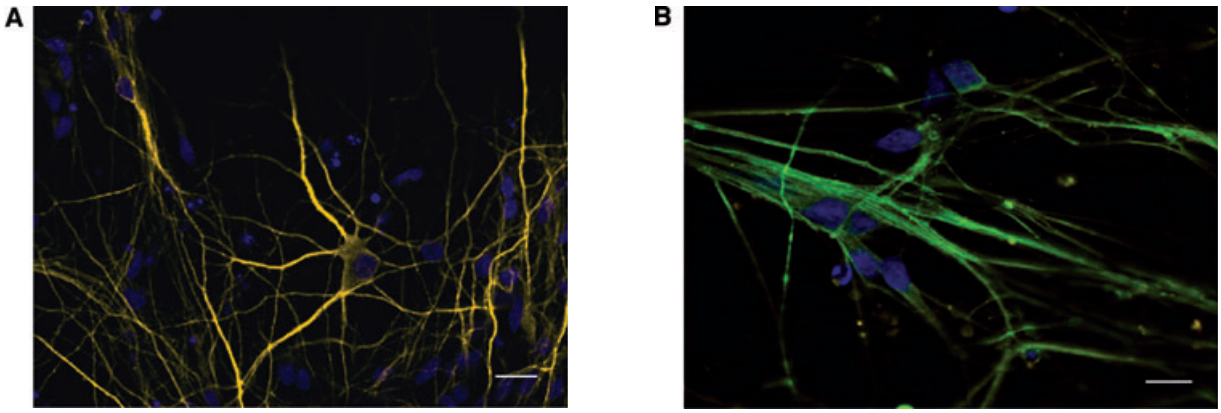

Fig. 2 Neuronal network of glutamatergic pyramidal-like neurons from hES cells. Human neurons derived from ES cells after 32 days of differentiation *in vitro*: they formed an extensive network, where the majority of cells are pyramidal-like neurons (**A**) expressing the neurotransmitter glutamate (**B**); staining for the neuronal marker tau (**A**—yellow) and glutamate (**B**—green); blue Hoechst 33342 nuclear staining. Scale bar: 20 μm.

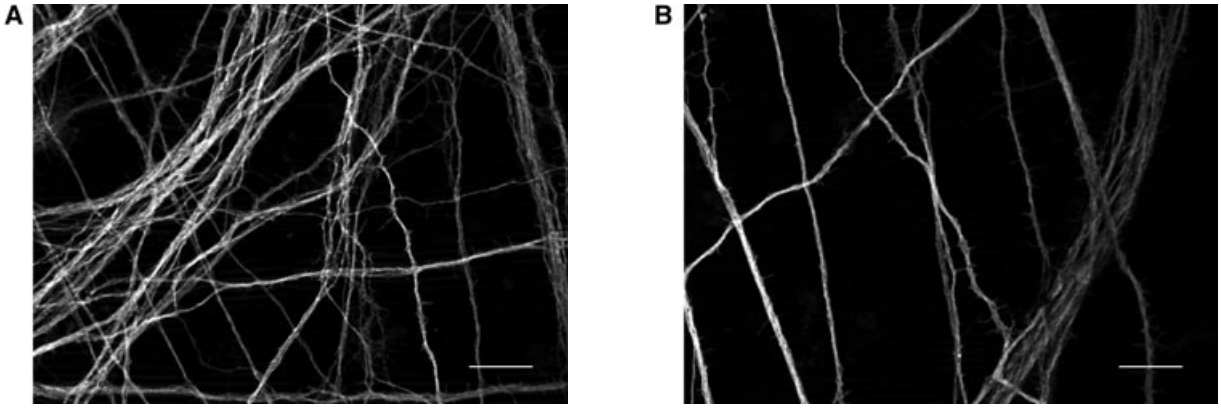

Fig. 3 Dendritic spine morphology of the neurons from hES cells. Human neurons derived from embryonic stem cells after 32 days of differentiation *in vitro*: the neurons formed very long projections with complex spine morphologies and different thickness (**A**, **B**); staining for the neuronal marker tau. Scale bars: 20 μm.

Acknowledgement

This study was supported by CNCSIS Romania (Project IDEI 626/2007).

Conflict of interest

The author confirms that there are no conflicts of interest.

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