Human Cortical Interneurons Take Their Time

Oscar Marín[1,*]
[1]Instituto de Neurociencias, Consejo Superior de Investigaciones Científicas & Universidad Miguel Hernández, Sant Joan d'Alacant 03550, Spain
*Correspondence: o.marin@umh.es
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In this issue, Maroof et al. (2013) and Nicholas et al. (2013) describe strategies for deriving cortical interneurons from human pluripotent stem cells. Functional maturation of these neurons occurs over a protracted period, which resembles normal human development, and suggests these cells may be useful for modeling brain development and disease.

The cerebral cortex consists of two main classes of neurons, glutamatergic projection neurons that are typically known as pyramidal cells, and gamma-aminobutyric acid-containing (GABAergic) interneurons. Interneurons only represent about one-fourth of the neurons in the cortex, but they fulfill important roles in regulating cortical function. In particular, interneurons modulate and synchronize the activity of pyramidal cells, which is critical for normal cortical functioning. Consistent with this idea, multiple lines of evidence directly implicate cortical interneuron dysfunction in several neurological and psychiatric illnesses, including epilepsy, autism, and schizophrenia (Marín, 2012).

The derivation of neurons from human pluripotent stem cells provides an excellent opportunity for modeling neurological and neuropsychiatric diseases in vitro. This approach also permits the generation of relatively homogenous populations of neurons, which facilitate their analysis and use for cell-based therapies. Thus far, efforts to differentiate human pluripotent stem cells to cortical neurons have primarily focused on generating pyramidal cells (Espuny-Camacho et al., 2013). In this issue of *Cell Stem Cell*, Maroof et al. (2013) and Nicholas et al. (2013) describe the generation of functionally mature cortical interneurons from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) using methods that recapitulate the normal development of this important population of neurons.

A substantial fraction of cortical interneurons, both in rodents and in humans, derive from the medial ganglionic eminence (MGE), a transient progenitor domain located in the ventral aspect of the telencephalon. The MGE also gives rise to interneurons that populate the striatum and other GABAergic neurons, including most projection neurons in the globus pallidus. MGE patterning involves at least two major steps, the specification of the telencephalic primordium by the transcription factor FOXG1 and the induction of ventral telencephalic fates by SHH. In response to this later signal, the MGE induces the expression of the transcription factor NKX2-1, which is essential for the specification of cortical interneurons (Sussel et al., 1999). NKX2-1 is also induced in the ventral diencephalon in response to SHH, but progenitor cells in this region lack expression of FOXG1 (Figure 1).

The MGE generates two major classes of cortical interneurons, characterized by the expression of the calcium binding protein Parvalbumin (PV) and the neuropeptide Somatostatin (SST), respectively. Expression of the transcription factor LHX6 is required downstream of NKX2-1 for the normal differentiation of PV+ and SST+ interneurons (Liodis et al., 2007). Interneurons migrate from the MGE to the cortex during embryonic stages in response to a variety of guidance cues. However, their functional maturation occurs postnatally. In humans, for example, PV+ interneurons are not fully differentiated until after birth. Understanding the mechanisms that control the protracted in vivo development of cortical interneurons is one of the many purposes of modeling their generation in vitro from human pluripotent stem cells.

The generation of specific classes of neurons in vitro involves several steps that progressively restrict the identity of pluripotent stem cells to the desired fate. Forebrain identity appears to constitute a default program of neural specification, and so the differentiation of hESCs or even hiPSCs naturally leads to rostral neural fates in the absence of additional morphogenetic signals. To enhance the production of telencephalic MGE-like precursors, Maroof et al. (2013) and Nicholas et al. (2013) designed similar, although not identical, small-molecule-based strategies (Figure 1). Both studies use a combination of several factors that promote the of SHH while maintaining a telencephalic identity. This is important, because SHH functions as a ventralizing factor throughout the CNS, and previous studies have shown that high SHH activation suppresses telencephalic markers such as FOXG1 (Fasano et al., 2010). To overcome this problem, both protocols improve FOXG1 induction through the inclusion of WNT signaling inhibitors in their differentiation cocktail. Using this method and an hESC line in which the gene encoding GFP has been inserted into the *NKX2-1* locus to facilitate the identification of ventral forebrain progenitors (Goulburn et al., 2011), both studies report the generation of MGE-like precursor cells (NKX2-1+/FOXG1+) with high efficiency. In addition, Nicholas et al. (2013) report similar results with an adult melanocyte-derived hiPSC line.

Maroof et al. (2013) show that the timing of SHH activation determines the fate of the neurons produced through the derivation of hESC progenitors. Thus, early exposure to SHH leads to hypothalamic NKX2-1+ fates, while late activation promotes the differentiation of the same hESC progenitors to GABAergic neurons with MGE-like features. Intermediate activation times favor the generation of cholinergic neurons, which are likely derived from telencephalic progenitors ventral to the MGE. These experiments

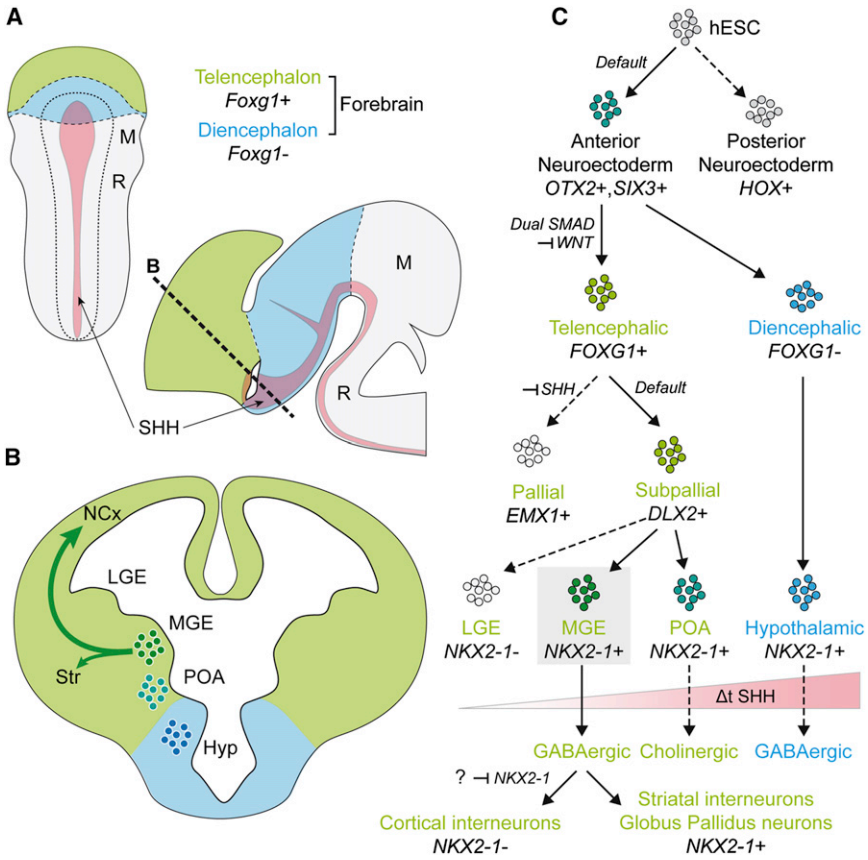

Figure 1. Normal Development Guides the Derivation of Human GABAergic Interneurons In Vitro

(A) Regionalization of the neural plate through the expression of specific transcription factors. Within the most anterior region of the neuroectoderm, the forebrain, the expression of FOXG1 distinguishes the primordium of the telencephalon (green) from the diencephalon (blue). Expression of SHH (pink) in the floor plate of the neural tube is important for the induction of forebrain fates and their subsequent ventralization.

(B) Schematic drawing of a coronal section through the forebrain in which different telencephalic and diencephalic domains are represented. Three populations of neurons are generated with the region of the ventral forebrain that express NKX2-1: GABAergic neurons derived from the medial ganglionic eminence (MGE), cholinergic neurons derived from the preoptic area (POA), and GABAergic neurons derived from the hypothalamus (Hyp). Most MGE-derived GABAergic neurons become cortical and, to a minor extent, striatal interneurons.

(C) Derivation of cortical GABAergic interneurons from human embryonic stem cells (hESCs). In the absence of additional signals, hESCs progress toward anterior neuroectodermal fates. Maroof et al. (2013) and Nicholas et al. (2013) use a dual SMAD protocol along with WNT inhibition to promote the specification of telencephalic neurons from hESCs. SHH influences the fate of these neurons, and so varying the precise timing of SHH action in vitro determines whether hESCs generate MGE-, POA-, or Hyp-like progenitor cells. Optimization of this parameter favors the generation of MGE-like progenitor cells (gray square), which gives rise to cortical and striatal interneurons. The specific production of cortical interneurons would require the postmitotic downregulation of NKX2-1, but the mechanism controlling this process in vivo is currently unknown.

M, mesencephalon; NCx, neocortex; R, rhombencephalon; Str, striatum.

are important because they reinforce the idea that the same signaling pathways that control the specification of different classes of neurons in vivo can be finely modulated to promote their differentiation in vitro.

Both studies analyzed the physiological properties of the neurons derived from MGE-like progenitors by culturing them along with cortical glial cells, and found that these cells have the characteristics of bona fide GABAergic interneurons. Nicholas et al. (2013) also found that a fraction of MGE-like progenitors obtained from human pluripotent stem cells downregulate the expression of NKX2-1 in culture. In mice, cortical interneurons downregulate the expression of NKX2-1 as they become postmitotic, while most striatal interneurons maintain it (Nóbrega-Pereira et al., 2008). This finding suggests that at least a fraction of the neurons derived from these experiments are indeed cortical GABAergic interneurons. This idea is further substantiated by an important set of transplantation experiments carried out by Maroof and colleagues that demonstrate that GABAergic neurons derived from hESCs migrate toward the cortex when transplanted into the MGE of mouse slices. All together, these experiments indicate that bona fide cortical GABAergic interneurons can be derived from human pluripotent stem cells. Nevertheless, it is worth emphasizing that these cultures are heterogeneous and contain other classes of GABAergic neurons, such as striatal interneurons. Additional factors will be necessary to enhance the preferential derivation of MGE-like progenitors fated to produce cortical interneurons.

One of the most intriguing findings of the studies by Maroof et al. (2013) and Nicholas et al. (2013) is the observation that GABAergic interneurons derived from human pluripotent stem cells require an extremely extended timeline to mature, which somehow mimics the normal development of these neurons in humans. In vitro, the protocol developed by Maroof and colleagues allows the identification of cells with neurochemical properties of cortical GABAergic interneurons and fairly mature physiological properties within 30 days of culture. However, Nicholas and colleagues show that these cells differentiate over an extremely protracted period of time when transplanted in vivo into the mouse cortex (Nicholas et al., 2013). Even 7 months after transplantation, most neurons derived from human pluripotent stem cells display relatively immature characteristics, and interneurons with the characteristics of PV+ fast spiking interneurons are nearly absent. Thus, the in vivo potential of cortical GABAergic interneurons derived from human pluripotent stem cells seems to be strongly limited by its own intrinsic program of maturation, which is very lengthy in humans. At this point, this is a serious limitation for the potential therapeutic application of these cells (Anderson and Baraban, 2012). Understanding the mechanisms that regulate the maturation of cortical interneurons in vivo may provide clues on how to accelerate the generation of fully

differentiated human interneurons from pluripotent stem cells.

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miR-34a Sets the "Sweet Spot" for Notch in Colorectal Cancer Stem Cells

Douglas J. Winton[1,*]
[1]Cancer Research UK Cambridge Institute, Li Ka Shing Centre, Robinson Way, Cambridge, CB2 0RE, UK
*Correspondence: doug.winton@cruk.cam.ac.uk
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Mechanisms underlying alternative modes of symmetric versus asymmetric stem cell fates are not well understood. In this issue of *Cell Stem Cell*, Bu et al. (2013) find that miR-34a acts as a toggle switch for Notch signaling that dictates binary symmetric and asymmetric fates in colon cancer stem cells.

Asymmetric cell division produces daughter cells with distinct fates and demonstrates a capacity for rapid spatial and temporal generation of phenotypic variation (Neumüller and Knoblich, 2009). The cellular machinery involved in delivering these outcomes and their impact on stem cell maintenance and differentiation has garnered much interest, but whether these mechanisms also affect fate choices in cancer stem cells has been less well-studied. In this issue of *Cell Stem Cell*, Bu et al. (2013) identify a role for the Notch-targeting microRNA miR-34a in asymmetric division of colon cancer stem cells (CCSCs).

miR-34a is a well-characterized microRNA that has been described as a tumor suppressor gene for many cancer types. Its widespread downregulation in cancers is well documented and has been attributed to locus deletion or epigenetic silencing (Vogt et al., 2011). Likewise, in various contexts, overexpression of miR-34a can induce apoptosis, senescence, differentiation, cell cycle arrest, and growth suppression in both in vitro and in vivo assays. One mechanism by which miR-34a promotes differentiation is inhibition of Notch signaling (Li et al., 2009). Notch signaling is frequently activated in colorectal cancers (CRCs) and is dysregulated directly by both epigenetic and genetic changes and indirectly by synergistic interactions with the Wnt pathway, which is also commonly activated in CRC. In addition, the Notch pathway has been previously identified to contribute to asymmetric cell division in stem cells. These well-characterized roles for Notch in directing lineage choice, asymmetric cell division, and colon cancer prompted Shen and colleagues to investigate the role of its upstream regulator, miR-34a, in asymmetric fate choice in CCSCs.

Using a panel of markers, the authors isolate a CCSC fraction and confirm its capacity to propagate the cancers in tumor xenograft assays. Importantly CCSCs are defined by low miR-34a levels, and overexpression of the microRNA inhibited tumor growth. To investigate modes of cell division in CCSCs, the authors borrowed from neural biology a "paired cell" assay, which allows the fate of single cells to be analyzed using combinations of differentiation and stem cell markers on the subsequent pairs that arise after cell division. Bu et al. identified asymmetric fates in around 20%–30% of naive CCSCs. The majority of the other cell pairs demonstrated symmetric fates into either CCSCs or non-CCSCs. Experimental elevation or reduction in the levels of miR-34a further promoted the co-occurrence of non-CCSC and CCSC symmetric fates, respectively. Strikingly, both manipulations reduce the frequency of asymmetric fates. In such pairs, daughters expressing the stem markers are more likely to reenter the cell cycle, suggesting one mechanism by which miR-34a may be exerting its tumor suppressive role in CRC.