EPILEPSY CURRENTS

Current Literature
In Basic Science

Recipes for Making Human Interneurons from Stem Cells Require Multiple Factors, Careful Timing, and Long Maturation Periods

Directed Differentiation and Functional Maturation of Cortical Interneurons from Human Embryonic Stem Cells.

Maroof AM, Keros S, Tyson JA, Ying S-W, Ganat YM, Merkle FT, Liu B, Goulburn A, Stanley EG, Elefanty AG, Widmer HR, Eggan K, Goldstein PA, Anderson SW, Studer L. *Cell Stem Cell* 2013;12:559–572.

Human pluripotent stem cells are a powerful tool for modeling brain development and disease. The human cortex is composed of two major neuronal populations: projection neurons and local interneurons. Cortical interneurons comprise a diverse class of cell types expressing the neurotransmitter GABA. Dysfunction of cortical interneurons has been implicated in neuropsychiatric diseases, including schizophrenia, autism, and epilepsy. Here, we demonstrate the highly efficient derivation of human cortical interneurons in an *NKX2.1::GFP* human embryonic stem cell reporter line. Manipulating the timing of SHH activation yields three distinct GFP+ populations with specific transcriptional profiles, neurotransmitter phenotypes, and migratory behaviors. Further differentiation in a murine cortical environment yields parvalbumin- and somatostatin-expressing neurons that exhibit synaptic inputs and electrophysiological properties of cortical interneurons. Our study defines the signals sufficient for modeling human ventral forebrain development in vitro and lays the foundation for studying cortical interneuron involvement in human disease pathology.

Functional Maturation of hPSC-Derived Forebrain Interneurons Requires an Extended Timeline and Mimics Human Neural Development.

Nicholas CR, Chen J, Tang Y, Southwell DG, Chalmers N, Vogt D, Arnold CM, Chen YJ, Stanley EG, Elefanty AG, Sasai Y, Alvarez-Buylla A, Rubenstein JLR, Kriegstein AR. *Cell Stem Cell* 2013;12:573–586.

Directed differentiation from human pluripotent stem cells (hPSCs) has seen significant progress in recent years. However, most differentiated populations exhibit immature properties of an early embryonic stage, raising concerns about their ability to model and treat disease. Here, we report the directed differentiation of hPSCs into medial ganglionic eminence (MGE)-like progenitors and their maturation into forebrain type interneurons. We find that early-stage progenitors progress via a radial glial-like stem cell enriched in the human fetal brain. Both in vitro and posttransplantation into the rodent cortex, the MGE-like cells develop into GABAergic interneuron subtypes with mature physiological properties along a prolonged intrinsic timeline of up to 7 months, mimicking endogenous human neural development. MGE-derived cortical interneuron deficiencies are implicated in a broad range of neurodevelopmental and degenerative disorders, highlighting the importance of these results for modeling human neural development and disease.

Commentary

Regenerative medicine, and in particular stem cells, bring the promise of curing diseases by providing replacements for worn-out or defective tissue and organs. For many epilepsies, the GABAergic inhibitory interneurons are either in too short supply or do not function properly. Thus, the balance of excitatory to inhibitory neurotransmission is tipped to overloaded excitation and runaway neural activity and seizures. Several anti-epileptic drugs function by increasing the concentration of the inhibitory neurotransmitter GABA to restore the balance between excitation and inhibition. However, drug therapy acts globally, having adverse effects on areas that do not require additional inhibition. A targeted strategy to replace the GABAergic interneurons at the seizure focus, such as the hippocampus in temporal lobe epilepsy, could provide a cure, permanently restoring the excitation/inhibition balance and leading to resolution of the seizures.

Neuroprogenitors, capable of proliferating in the tissue culture dish, have been studied for the past 25 years. Neural stem cells and differentiated neurons have been obtained from human pluripotent stem cells (hPSCs), including embryonic stem

Epilepsy Currents, Vol. 13, No. 6 (November/December) 2013 pp. 266–268

cells (ESCs), adult stem cells (SCs) and induced pluripotent stem cells (iPSCs). Yet very few neurons were obtained from the previous in vitro techniques—1 to 5 percent of the total population—and the neuronal cell types were often based on general phenotypic markers. Many of molecular mechanisms required in the ontogeny of specific neuronal types are unknown. From the initial lineage restriction to a neural progenitor cell capable of producing neurons or glia, cerebral cortical and hippocampal GABAergic interneurons must be specified to express anterior forebrain molecular markers, and then further restricted to the ventral forebrain lineage. Finally, because ventral forebrain neural progenitors have the potential to become GABAergic interneurons, as well as cholinergic interneurons or GABAergic striatal projection neurons, final differentiation steps into GABAergic interneurons must occur, followed by migration to correct cortical area and incorporation into functional neural circuits. From basic science research, some of the steps in the ontogeny from stem cells to adult GABAergic interneurons have been defined in intact rodent models, but many questions remain in the human.

Three major challenges have delayed translation of the stem cell technology into the clinic. First, sufficient quantities of neural stem cells must be derived from the undifferentiated stem cells. Secondly, the molecular determinants of the inhibitory GABAergic neuronal lineage must be known and applied to stem cell cultures to obtain mature neurons with specific properties and function. Thirdly, the derived cells must be safe and remain within their defined lineages and not become oncogenic or otherwise harmful. Two publications in *Cell Stem Cell* from the Studer and Kriegstein laboratories address the challenges and make major strides in bringing stem cells closer to human therapies (1, 2). Using small molecule inhibitors of the WNT (wingless-type) ligands and the intracellular SMAD (derived from the combination of the *sma* (*Caenorhabditis elegans)* and *Mad* (*Drosophila)* transcription factors proteins, which direct gene transcription and cell fate, both groups demonstrated robust expression of GABA neuroprogenitor transcription factor (*NKX2.1,* as determined using an *NKX2.1-GFP* reporter gene) and > 85% co-localization with other ventral forebrain neural specific proteins, in multiple hESC and hIPSC lines. These protocols with small molecule drugs can be easily adapted to commercial applications and good manufacturing practices. The promising results with multiple embryonic and adult hPSCs suggest a universal protocol to convert stem cells to large numbers of ventral forebrain neural stem cells.

Converting ventral forebrain neuroprogenitors into the unique subtypes of cortical GABAergic interneurons found in the adult represented a higher hurdle. In vivo, the GABAergic interneurons must migrate from their birthplace into the emerging cerebral cortex and hippocampus. In the rodent, the majority of the GABAergic interneurons are born in the ventral forebrain (3, 4). Current evidence implies that a substantial fraction of human GABAergic interneurons also derive from ventral sources, while the rest arise along with the glutamatergic neurons in the developing cerebral cortex (5, 6). The migration potential of the hESC-derived NKX2.1-GFP cells was evaluated by grafting them into embryonic mouse explant cultures and transplanting into neonatal mouse cortex (2). The authors discovered a critical period of differentiation that generated GABAergic cells capable of migrating to the cortex, along with culture conditions that optimized the alternative lineages of potential striatal medium spiny projection neurons and hypothalamic neurons.

Once the young GABAergic interneurons reach the cerebral cortex and settle into the appropriate layers, the cells mature and integrate into circuits. Long-term cultures (30 weeks after differentiation) expressed GABA and markers of mature interneurons, including calbindin, calretinin, and somatostatin. But very few cells showed parvalbumin, which marks the fast-spiking interneurons that are commonly lost in seizures (1). The firing properties of the NKX2.1-GFP cells matured in culture, corresponding to the emergence of biochemical markers. Previous studies have shown that maturation occurs in the presence of cell types present in the postnatal brain, including glial cells, and possibly neurons. Co-cultures with mouse glial cells or mixed perinatal mouse neuronal and glial cells elicited pre-synaptic and post-synaptic spontaneous firing, indicating that the hPSC-derived cells can assemble into functional networks (1). NKX2.1-GFP cells grown on feeder layers derived from hESCs appeared to be less mature, based on electrophysiological measurements, than those grown on mouse feeder layers, indicating local environment and perhaps glial composition influences development (2). Again, very few parvalbumin neurons were found and only after extended time in culture. Finally, the NKX2.1-expressing cells were transplanted into mouse cerebral cortex to determine if in vivo factors could guide maturation (1, 2). The cells dispersed and maintained expression of GABA, but parvalbumin-expressing cells were rarely observed, even after 7 months after transplantation. Overall, these multiple experiments point to a protracted time course for final maturation of the GABAergic interneurons, especially the parvalbumin subtype.

Transplantation of embryonic mouse GABAergic neuroprogenitors into postnatal and adult mouse brains can rescue interneuron defects and seizures (7, 8), but transplantation of pluripotent stem cells (hPSCs) leads to tumor formation. In fact, transplantation of the NKX2.1-GFP cells into mouse brain had 100% incidence of tumors (1). The tumor incidence dropped to 0% when the injected cells were restricted to the cells that expressed a specific cell adhesion molecule (PSA-NCAM). Perhaps the expression of PSA-NCAM indicates a checkpoint in the ability to revert to the undifferentiated state. Avoiding the formation of tumors is crucial to the success of stem cell transplants for human therapies.

In conclusion, the groups led by Kriegstein and Studer have overcome several of the hurdles in generating GABA interneurons for use in human therapies. Previous methods were unable to generate sufficient numbers of replacement cells for transplantation into patients or preclinical drug testing applications. The methods described by Kriegstein and Studer can produce substantial fractions of specific neurons that are capable of maturing into neurons with characteristics that approach adult cells, representing a major step in translating stem cell biology into regenerative medicine.

by Elizabeth M. Powell, PhD

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