Cell

Cerebral Organoids in a Dish: Progress and Prospects

Marina Bershteyn[1,2] and Arnold R. Kriegstein[1,2,3,*]
[1]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, 35 Medical Center Way, San Francisco, CA 94143, USA
[2]University of California, San Francisco, San Francisco, CA 94143, USA
[3]Department of Neurology, University of California, San Francisco, San Francisco, CA 94143, USA
*Correspondence: kriegsteina@stemcell.ucsf.edu
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A three-dimensional culture of cortical tissues derived from pluripotent stem cells offers an opportunity to model human brain development and disorders. In a recent issue of *Nature*, Lancaster et al. describe a new method for generating cerebral organoids in a dish and use it to model microcephaly.

Just a little more than 6 years ago, it would have seemed like science fiction to take human skin cells and create parts of an embryonic cortex. But today, thanks to the tremendous progress in our ability to manipulate cell identity, it is possible to reprogram adult skin fibroblasts into induced pluripotent stem cells (iPSCs) (Takahashi et al., 2007) and coax them into becoming a broad range of tissues. Under the right conditions, PSCs have a remarkable capacity to self-organize and develop into recognizable three-dimensional structures resembling miniature organs, including the intestine, thyroid, retina, or cortex (reviewed by Sasai, 2013; Figure 1). These in vitro preparations complement in vivo model organisms and help to elucidate principles of organ development and mechanisms of genetic diseases.

The cerebral cortex is one of the most intricate and complex organs in the body. Its developmental origin, however, stems from a single layer of neuroepithelial progenitors that give rise to all the other cell types in the cortex. Several laboratories have demonstrated that PSCs grown in vitro have an intrinsic tendency to form polarized neuroepithelial structures with striking similarities to progenitor zones of the embryonic cortex (Eiraku et al., 2008; Gaspard et al., 2008; Mariani et al., 2012). In a recent issue of *Nature*, Lancaster et al. (2013) describe a method for growing three-dimensional (3D) neural tissue from human PSCs. They use this method to model microcephaly—a developmental disorder characterized by severely reduced brain size. Their approach builds on previous methods but incorporates new features, including modified culture conditions and use of a spinning bioreactor. The resulting cerebral organoids reach up to 4 mm in size and contain more elaborate structures of the human embryonic brain than what has been shown previously.

Lancaster et al. (2013) initiate formation of cerebral organoids in embryonic stem (ES) cell medium with low levels of basic fibroblast growth factor and then transfer 3D aggregates into neural induction medium. In contrast to many current directed neuronal differentiation protocols, this method does not involve use of the SMAD inhibitors that promote neuroectoderm while suppressing mesoderm and endoderm (Chambers et al., 2009). These culture conditions are expected to produce spontaneous differentiation into neural as well as nonneural cell types. Indeed, multiple regions within the organoids lack neuronal identity based on expression of general markers.

The spinning bioreactor is used to improve nutrient absorption in growing organoids and enables formation of longer continuous neuroepithelial-like zones, instead of the smaller rosette structures obtained with other protocols. Expanded neuroepithelial regions consist of polarized radial glia-like stem cells that surround a fluid-filled cavity resembling the lateral ventricle in the developing brain. Because these cavities are fairly large, the authors are able to use intraventricular electroporation methods similar to what is done in mouse embryos in utero and achieve specific labeling of the neural stem cells. This is a useful approach for characterizing the behavior of the founder cells and their progeny, and it can be used to genetically manipulate the cells.

In the developing forebrain, inhibitory interneurons are born in the ventral parts of the telencephalon and migrate tangentially into the dorsal cortex. The same type of migration between ventral and dorsal regions appears to be recapitulated in a subset of the cerebral organoids produced by Lancaster et al. Specifically, there is a correlation between regions with ventral forebrain identity and the presence of Calretinin-expressing cells oriented toward dorsal regions. Although the evidence is indirect, as it is based on a single time point and marker, it is an example of how this model system can be used in the future to study signaling between different cell types within brain-like tissues generated in a dish.

Despite the presence of multiple brain-like regions, the authors acknowledge that cerebral organoids do not fully model the organization of the brain. Formation and juxtaposition of different regions is random and lacks the overall structure that develops in vivo. Moreover, cortical lamination is incomplete, with only two layer-specific neuronal subtypes produced, suggesting that some key developmental cues are missing. This has been an ongoing challenge for other aggregation-based methods, although neurons of all six-layer identities have been successfully generated using

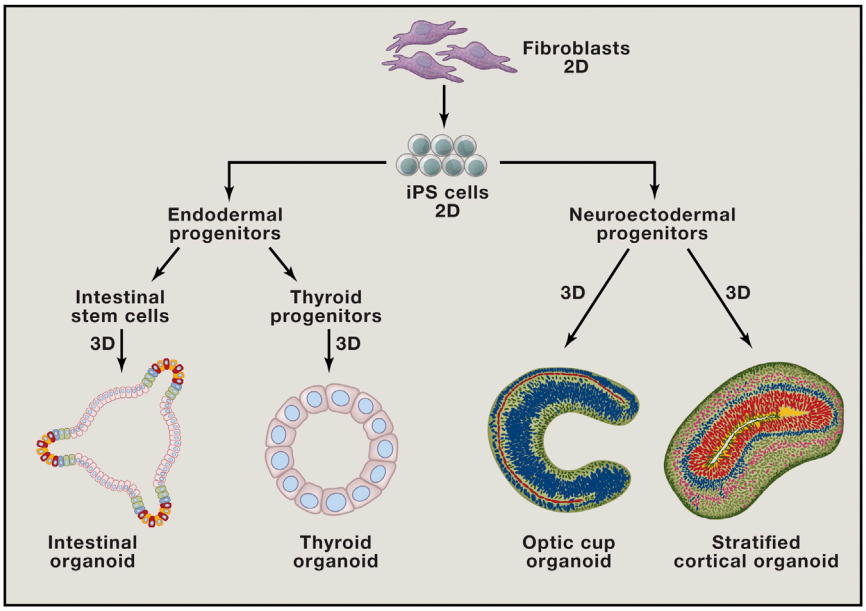

Figure 1. Generation of 3D Endodermal and Ectodermal Organoids from Skin Fibroblasts
Reprogramming of fibroblasts into induced pluripotent stem cells (iPSCs) enables in vitro differentiation into tissue-specific progenitors, such as intestinal stem cells, thyroid, or neuroectodermal progenitors. When grown under the right conditions in suspension, these progenitors self-organize into 3D tissues resembling the intestine, thyroid, optic cup, or cerebral cortex.

2D culture methods (Espuny-Camacho et al., 2013).

Given that in-vitro-generated brain tissues are still a simplified haphazard approximation of their in vivo counterparts, what is the advantage of using human stem cell models rather than in vivo mouse models? One compelling argument will come from a clear demonstration that stem-cell-derived tissues recapitulate distinguishing features of human brain development. For example, unlike mice, humans have an expanded outer subventricular zone (oSVZ) made up of neurogenic radial glia-like cells (oRGs) and transit-amplifying cells that contribute significantly to increased cortical size and complexity (Hansen et al., 2010). It has been shown that oRG cells can be produced in vitro from human PSCs (Shi et al., 2012). Importantly, Lancaster et al. (2013) extend these observations by showing that the oRGs reside in oSVZ-like progenitor regions within the cerebral organoids, much like they do in vivo. In contrast, mouse ES cells differentiated using a similar protocol fail to generate oSVZ-like regions or oRGs. Provided that the generation of human-specific progenitor zones is a robust feature of this system, this model may serve as a valuable in vitro platform for studying the molecular mechanisms that regulate human oRG development.

Another advantage of using human iPSCs is the potential to gain new insight into brain disorders in the most relevant genetic context. For example, it is unknown why mutations that lead to microcephaly in people do not severely reduce the size of a mouse brain. In their iPS model of microcephaly, Lancaster et al. (2013) identify a dramatic reduction in the founder population of neuroepithelial stem cells, coupled with premature neuronal differentiation. Although this phenotype is consistent with the prevailing theories about microcephaly pathogenesis based on mouse studies, the mechanisms behind it are still uncertain. It would be interesting to compare how the same mutations will affect mouse cerebral organoids in order to understand the discrepancy between minor phenotypes in mice and severe phenotypes in humans.

In conclusion, with the growing number of protocols for generating cortical neurons in vitro, it is becoming clear that there are multiple approaches to model human cortical development. The protocol developed by Lancaster et al. (2013) provides advantages over previous aggregation methods, including favorable conditions for the formation of an oSVZ-like progenitor zone. In addition, spontaneous coemergence of different brain-like regions within a single organoid may yield an in vitro platform for elucidating mechanisms of brain patterning. Their work is an important step toward development of more refined protocols that will eventually achieve better organization of tissues along the dorsal-ventral and anterior-posterior axes. In time, the ability to create more complete cerebral organoids in a dish may come closer to reality. When that happens, besides having the convenience of in vitro models of brain development and disease, there will be a whole new set of ethical and philosophical issues to contemplate.

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