Uncovering cell biology in the third dimension

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ABSTRACT

Developmental biology has long benefited from studies of classic model organisms. These model systems have provided the fundamental understanding of general principles of development, as well as insight into genes and signaling pathways that control unique aspects of cell fate specification and tissue morphogenesis. Because human brain development cannot be studied in vivo, scientists have relied on these model systems to study basic principles underlying the development of this complex organ as many of these genes and signaling pathways play conserved roles in human development. However, recent studies have shown species-specific signatures in neurodevelopment such as the transcriptome of outer-radial glia, suggesting use of a human-derived model remains imperative. Over the past decade, human stem cell-derived brain organoids have emerged as a biologically relevant model system to study normal human brain development and neurological diseases. Here, we provide a historical perspective of this emerging model system, discuss current systems and limitations, and propose that new mechanistic insight into cell biology can be revealed using these three-dimensional brain structures.

INTRODUCTION

Brain organoids are three-dimensional (3D) self-assembled structures that can be formed in vitro from human pluripotent stem cells (hPSCs) that resemble the cellular organization and transcriptional and epigenetic signature of a developing human brain up to ~24–25 wk postconception as compared with fetal brain tissue (Camp et al., 2015; Quadrato et al., 2017; Amiri et al., 2018; Kanton et al., 2019; Paśca et al., 2019; Velasco et al., 2019). By culturing in circulating medium, brain organoids develop a neuroepithelium that can mature to generate neural stem cells with characteristic architecture and to generate progenitor zones (ventricular zone, subventricular zone, and subsequently outer subventricular zone), all identifiable by cell type-specific molecules such as transcription factors PAX6 and SOX2 and apically polarized N-cadherin. When cultured for weeks to months, organoids generate multilayered structures around a central lumen, patterned in an “inside-out” manner (referring to the direction of progenitor differentiation and migration in the cortex from the ventral side to the cortical plate, with neurons in Layer II being younger than Layer VI neurons). The neurons generated from these systems form functional synapses, secrete relevant neurotransmitters, and express cell-type specific markers (Kanton et al., 2019; Trujillo et al., 2019; Velasco et al., 2019). Thus, brain organoids have become useful tools to model early to midgestational human brain development. In addition, patient-derived brain organoids have successfully been used to model human neurodevelopmental disorders such as Timothy syndrome and autism spectrum disorder (ASD) (Mariani et al., 2015; Birey et al., 2017). While the field has grown immensely, we are just at the initial stages of uncovering how far brain organoid technology can develop. In this perspective, we summarize advancements in this field, speculate on future directions for the technology, and focus on the potential applications of brain organoids for the study of basic cell biology.

PERSPECTIVE

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Abbreviations used: ASD, autism spectrum disorder; BBB, blood–brain barrier; hPSC, human pluripotent stem cell; EB, embryoid body; iPSC, induced pluripotent stem cell; SFEBq, serum-free floating culture of embryoid body-like aggregates with quick reaggregation.

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HISTORICAL PERSPECTIVE ON BRAIN ORGANOIDS

Studies using classic model organisms have provided crucial genetic information of key signaling pathways underlying human development. While in vitro 2D culture systems have been useful platforms to test and manipulate these signaling pathways, the development of hPSC-derived 3D organoids has allowed the field to start integrating other developmental factors such as complex tissue architecture, dynamics, as well as some of the signaling gradients found in vivo. This innovation was spurred on by advancements in human stem cell culture and reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) (Thomson, 1998; Takahashi and Yamanaka, 2006; Yamanaka et al., 2007). Guided methods for the formation of 3D neuronal structures derived from human stem cells were pioneered by the Sasai group (Eiraku et al., 2008, 2011; Danjo et al., 2011; Kadoshima et al., 2013; Muguruma et al., 2015). These polarized cortical tissues were electrophysiologically functional and transplantable into mice (Eiraku et al., 2008). Importantly, the SFEBq (serum-free floating culture of embryoid body (EB)-like aggregates with quick reaggregation) method (Kadoshima et al., 2013) is the basis of many of the current unguided and guided organoid methodologies (Qian et al., 2019). At the inception of the organoid field, intestinal organoids were generated from single intestinal stem cells derived from mouse crypts (Sato et al., 2009). Much like brain organoids, these intestinal organoids self-organized to form a 3D architecture that closely resembles the in vivo structure and were later also derived from iPSCs using a temporal series of growth factor treatments that mimicked embryonic intestinal development (Spence et al., 2011). These previous studies on intestinal organoids paved the way for much of the technology and methods later adapted to advance brain organoid generation, which recapitulated the structure and function of multiple brain regions.

CURRENT SYSTEMS TO GENERATE BRAIN ORGANOIDS

The cerebral organoid model developed rapidly because of the propensity of cells to self-assemble into this structure without the addition of specific patterning molecules. When cells are grown in suspension in serum-free media, they develop into an EB. These EBs can then be embedded in Matrigel, an extracellular matrix composite that supports organoid growth and progenitor cell polarization. Without the addition of exogenous soluble factors, EBs can stochastically develop into unguided organoids containing a diverse array of neural cell types including dorsal and ventral forebrain, retina, hippocampus, midbrain, and hindbrain (Lancaster et al., 2013; Lancaster and Knoblich, 2014; Camp et al., 2015; Quadrato et al., 2017) (Figure 1).

Through the use of specific patterning molecules, organoids can also be directed to differentiate into specific brain regions (guided organoids). For example, EBs can be generated as described above with the addition of SMAD and Wnt inhibitors to promote dorsal forebrain identity (Birey et al., 2017). Midbrain-like organoids containing dopaminergic neurons have been generated using sonic hedgehog and FGF8 (fibroblast growth factor 8) patterning (Jo et al., 2016). If IWP-2 (Inhibitor of WNT) and SAG (Smoothened Agonist) are also added, the organoids can adopt ventral forebrain identity (Birey et al., 2017). It is important to note that while the organization around the lumen is highly reproducible (Velasco et al., 2019), unlike human development, several lumens of varying size are generated using current protocols. Recent developments in micropattern technology provide a standardized system to dissect the earliest processes of human neural induction and morphogenesis within a single lumen (Haremaki et al., 2019).

Organoids have also been generated for structures such as the optic cup and the spinal cord. Optic cup organoids exhibit

FIGURE 1: Time-line representation of significant advancements in the generation of brain organoids. SFEBq: serum-free floating culture of EB-like aggregates with quick reaggregation.
apical-basal polarity and recapitulate the evagination of the epithelial vesicle and expression of neural retina markers such as CHX10 and SIX3. These organoids also demonstrate interkinetic nuclear migration, a hallmark of early neurogenesis that has been difficult to study in vitro. Protocols for organoids modeling ventral, intermediate, and dorsal spinal cord identity called 3-DiSC (3D-induced spinal cords) were recently developed (Ogura, Sakaguchi, et al., 2018). The spinal cord organoids were ventralized by changing the concentration of sonic hedgehog agonist and dorsalized by the addition of BMP4 treatment. These organoids generated spinal motor neuron and spinal interneuron subtypes, as well as a roof plate-like organizing center that has not been produced with other organoid protocols.

Further breakthroughs have utilized multiple organoid systems to model complex cell biology such as migration. For example, separately patterned dorsal and ventral forebrain organoids were integrated into what has been called assembloids (Birey et al., 2017). When these region-specific organoids are assembled, GABA-ergic interneurons migrate from the ventral organoid into the dorsal organoid, resembling the in vivo process of interneuron precursor migration (Birey et al., 2017). Later work generated assembloids by combining independently differentiated cortical organoids and medial ganglionic eminence organoids (Xiang et al., 2017). This model also mimics the developmental migration of MGE-derived interneurons into the cortex (Xiang et al., 2017). Beyond migration, the assembly of organoids may allow for the investigation of complex neural circuits that connect various brain regions.

Alongside advances in differentiation protocols, bioengineering technology has improved many aspects of brain organoid production. For example, in order to promote long-term culture, organoids have been grown in spinning bioreactors to improve nutrient and waste product exchange to and from the densely packed structures. These miniature bioreactors are compatible with multi-well plates, which decreases costly culture media and increases reproducibility and throughput (Qian et al., 2018; Romero-Morales et al., 2019; Velasco et al., 2019). Other examples include the use of embedded microfilaments within the organoid and culture at an air-liquid interface to enhance cortical plate formation (Giandomenico et al., 2019). It is expected that engineering principles will help enhance brain organoid platforms in the future.

Despite the great advances in the generation of structures that resemble human development, some challenges remain. For example, all in vitro brain organoid platforms lack perfusable microvasculature. Currently, most vascularization approaches rely on bio-printing or coculturing organoids with a monolayer of endothelial cells (Pham et al., 2018; Grebennyuk and Ranga, 2019), but these strategies do not recapitulate the capillary structures seen within the brain. The transcriptomic profile of organoids also resembles that of midgestation (Camp et al., 2015; Quadrato et al., 2017; Amir et al., 2018; Kanton et al., 2019; Velasco et al., 2019), and while this is an impressive improvement from 2D cultures, there are still limitations to model late-onset neurological diseases. Although the field has been able to produce many different brain regions independently, the ability to mimic in vivo development with temporal and structural resolution may require morphogen gradients and localized application of small molecules.

USING ORGANIOIDS TO REVEAL BASIC CELLULAR BIOLOGICAL MECHANISMS

While the potential of brain organoids to model disease has motivated many investigations, one powerful application that has remained somewhat overlooked is the ability to uncover basic biological mechanisms underlying brain development in the context of a temporally and spatially organized 3D tissue. In addition, the widespread use of genome editing in hPSCs has made it possible to introduce fluorescent tags at the endogenous locus of fundamental genes allowing for the visualization of cellular processes in live cells within the complex brain architecture. It is remarkable that these technological and biological advancements have now opened the door for mechanistic studies to be conducted in systems that are close to the physiology and anatomy of the human brain. While many areas of biology could benefit from these systems, we are focusing our discussion into three potential lines of investigation.

1. Brain vascularization and blood–brain barrier (BBB) development

Brain vascularization is initiated by a perineural vascular plexus, where endothelial cells sprout into brain tissue, recruit pericytes, and form the BBB. There is evidence that this process is spatially coordinated, leading to the development of region-specific BBB identity that may reflect the specialized functions of specific neuron subtypes (Vasudevan et al., 2008). While isolated 2D cultures could shed light on this cross-talk, they are not likely to provide as much insight into developmental dynamics involving explicit 3D behaviors like angiogenesis and organization of vascular structures. On improvement of endothelial cell integration, and when coupled with cellular reporters and single-cell transcriptomics, organoids could provide a valuable resource for studying mechanisms of human brain vascularization and regional specification, which may have significance for neurodegenerative diseases that involve BBB dysfunction (e.g., Alzheimer’s disease), although other facets of aging may need to be incorporated into these models to reliably recapitulate such phenotypes (Cakir et al., 2019).

2. Neuron-glia biology

Traditional 2D hPSC-derived neuronal cultures have been instrumental for fundamental studies of individual cell behaviors. Further, coculture systems complemented with animal models have recently expanded on the basic understanding of how neurons and astrocytes exert noncell autonomous effects to promote survival and function (Ioannou et al., 2019). While the many supportive roles of astrocytes (e.g., astrocyte-neuronal lactate shuttle, control of extracellular pH, and control of cerebral blood flow) have been studied for decades, astrocytes have also been shown to uptake and release neurotransmitters via calcium-dependent vesicular release, suggesting that they play an integral role in neural circuits (Allen and Barres, 2009). Because of their cellular diversity and neuronal network activity (Quadrato et al., 2017), organoids provide a platform to study the interplay of neurons and glia within the dynamic context of specific brain regions as well as with the temporal resolution of human development, allowing for new paradigms to be discovered. The ability to generate complex human neural circuits that can be stimulated by physiological conditions (Quadrato et al., 2017) could also provide insight into the mechanisms underlying high-order functions and neurotransmission.

Along with astrocytes, other nonneuronal cells such as oligodendrocytes, ependymal cells, and choroid plexus cells have been identified in the diverse cellular population of organoids (Jo et al., 2016; Matsui et al., 2018; Yoon et al., 2019). The presence of these cells allows for the investigation of unique cellular mechanisms that occur during neurodevelopment such as interkinetic nuclear migration and myelination (Madhavan et al., 2018), although in some cases the desired cell type is relatively rare (e.g., choroid plexus epithelial cells) and improved differentiation methods may be necessary to
increase yields for targeted studies. Given that ependymal and choroid plexus cells play a central role in the blood–CSF barrier, the crucial process of ion homeostasis and osmotic pressure regulation could possibly be investigated using organoid systems as well.

3. Mitochondrial biology and metabolism

Mitochondrial function is crucial for maintaining homeostasis of highly metabolic tissues. The brain consumes nearly 20% of the oxygen and calories from the whole body, while representing ~2% of its total weight (Picard and McEwen, 2014). While the exact underlying mechanisms responsible for the complex cognitive capacity of the human brain remain elusive, it is not surprising that mitochondrial health and metabolism are linked to the normal development of the human central nervous system. Several studies in both humans and other animals have linked defects in mitochondrial fusion and fission and in mitochondrial metabolism with complex disorders such as Leigh syndrome, neurodevelopmental disorders such as ASD, and with neurodegenerative diseases such as Alzheimer’s and amyotrophic lateral sclerosis.

Despite this irrefutable link between mitochondrial dysfunction and human disease, the regulation of mitochondrial morphology and function in the context of the human brain remains unexplored. Seminal studies have relied on yeast, cultured mammalian cells, and mice (Meeuseen et al., 2006; Khacho et al., 2016; Ioannou et al., 2019) to improve our understanding of the basic principles and molecular mechanisms governing mitochondrial morphology and function. However, based on its unique metabolic requirements, the human brain may have novel modes of mitochondrial modulation that could not be gleaned from simpler organisms. Brain organoids, models, coupled with gene editing, classical biochemistry, high resolution microscopy, and proteomics, could provide the tools required to embark on this quest to uncover the contribution of mitochondria and metabolism to human brain development. Moreover, a better understanding of mitochondrial biology and interorganellar communication in early human brain development could provide insight into the molecular details underlying neuronal specification, migration, and maturation.

While organoids are not necessarily brains in a dish, they serve as an indispensable model system to uncover fundamental mechanisms of basic cell biology, human neurodevelopment, and neurological pathology. There have been tremendous advances in the past decade that continue to push the boundaries of mimicking in vivo human brain development. The emergence of brain organoid systems and the advancement of biomedical engineering have placed the field in the unique position to shed light on the basic molecular mechanisms involved in human brain development. Evolutionary-focused studies are emerging on understanding what distinguishes humans from other species, which is thought to reside on the unique features of brain development, in particular the complexity of neural circuits that underlie our remarkable cognitive abilities (Giandomenico and Lancaster, 2014; Muchnik et al., 2016).

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