



Minireview

Past, Present, and Future of Brain Organoid Technology

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Brain organoids are an exciting new technology with the potential to significantly change our understanding of the development and disorders of the human brain. With step-by-step differentiation protocols, three-dimensional neural tissues are self-organized from pluripotent stem cells, and recapitulate the major milestones of human brain development *in vitro*. Recent studies have shown that brain organoids can mimic the spatiotemporal dynamics of neurogenesis, the formation of regional neural circuitry, and the integration of glial cells into a neural network. This suggests that brain organoids could serve as a representative model system to study the human brain. In this review, we will overview the development of brain organoid technology, its current progress and applications, and future prospects of this technology.

Keywords: brain disorder, brain organoid, neurodevelopment, pluripotent stem cell, three-dimensional culture

INTRODUCTION

Understanding human brain development and brain disorders is one of the most fascinating challenges in biology. In addition to the daunting complexity of the human brain, difficulties in accessing human brain tissue have hampered our efforts to decipher the secrets of the human brain. Post-mortem or surgically removed human brain samples have several disadvantages, including such as variability in genetic and en-

vironmental background, and inconsistency in tissue processing and preservation. It is also hardly possible to work with live human brain tissues, which is important for studying cell biological principles of human brain development. Therefore, animal model organisms (e.g., a mouse) have been widely used to examine the development and function of the brain. However, there are quite a lot of differences between the development of a human and that of a mouse brain. For example, most of the radial glial cells (RGCs), primary neural stem cells, are located in the ventricular zone of the mouse developing neocortex. However, not only ventricular radial glial cells (vRGCs), but also an abundant population of outer radial glial cells (oRGCs) in the outer subventricular zone (OSVZ) contribute to the evolutionary expansion of the neocortex in humans (Lui et al., 2011). The division patterns of neural stem cells at the specific stages of brain development exhibits major differences between mice and humans (Homem et al., 2015). Therefore, we need a new model system that can better represent the characteristics of the development of the human brain.

Human pluripotent stem cells (hPSCs), including embryonic stem cell (Thomson et al., 1998) and induced pluripotent stem cell (iPSC) (Takahashi et al., 2007), have enabled unprecedented opportunities to study the development of the human brain and the pathobiology of human disorders (Yamanaka, 2012). Human iPSCs were generated from human somatic skin cells by ectopic expression of stem cell transcription factors (Takahashi et al., 2007; Yu et al., 2007). In the past decade, hPSC-derived systems have been widely used

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for variety of researches on human diseases. For example, by using iPSCs derived from patients, it is possible to access a non-invasive, patient-specific and ethically sustainable model system. In addition, powerful genome editing tools such as the CRISPR/Cas9 system enable scientists to freely modify the genetic information of hPSC (Hockemeyer and Jaenisch, 2016; Kim et al., 2019b). Currently, *in vitro* differentiation protocols for inducing hPSCs to differentiated into various types of human neural cells are available (Oh and Jang, 2019; Tao and Zhang, 2016). These protocols, which are based on a two-dimensional (2D) culture system, have several advantages. First, a monolayer culture system enables the uniform accessibility to growth/differentiation factors, which helps to acquire a relatively homogeneous population of differentiated cells with high purity. Second, it is scalable to a minimal culture size for high-throughput screenings of small molecules and a genome-wide CRISPR/Cas9 library. Third, the flatness of monolayer cells makes live-imaging experiments more feasible.

Despite these advantages, 2D culture also has several limitations for modeling the complex human brain development. First, cell-to-cell or cell-to-extracellular matrix interactions, which regulate important steps of neurodevelopment, are largely missing in a monolayer culture. Second, the spatial

gradient of growth factors, patterning factors, nutrients, and gas exchange are critical for regional specification of the human brain, which is a challenge to model with a monolayer system. Third, a planar culture cannot recapitulate certain important cellular properties, such as cell polarity and guided cell migration. Therefore, it is necessary to develop a better model system that better reflects a genuine environment for human brain development.

Brain organoids are organ-like three-dimensional (3D) tissue cultures containing brain-specific cell types derived from PSCs (Lancaster and Knoblich, 2014; Qian et al., 2019). In this review, we introduce this emerging new technology, brain organoids, as an alternative model system to investigate the development and the disorders of the human brain. We will also discuss the history, current progress, potential applications and limitations of brain organoid technology.

DEVELOPMENT OF THREE-DIMENSIONAL BRAIN ORGANOID SYSTEMS

Self-organization, the intrinsic and spontaneous ability to form specific cellular structures without external factors, plays a key role in the formation of organs (Werner et al., 2017). For example, adhesion proteins on a cell surface drive au-

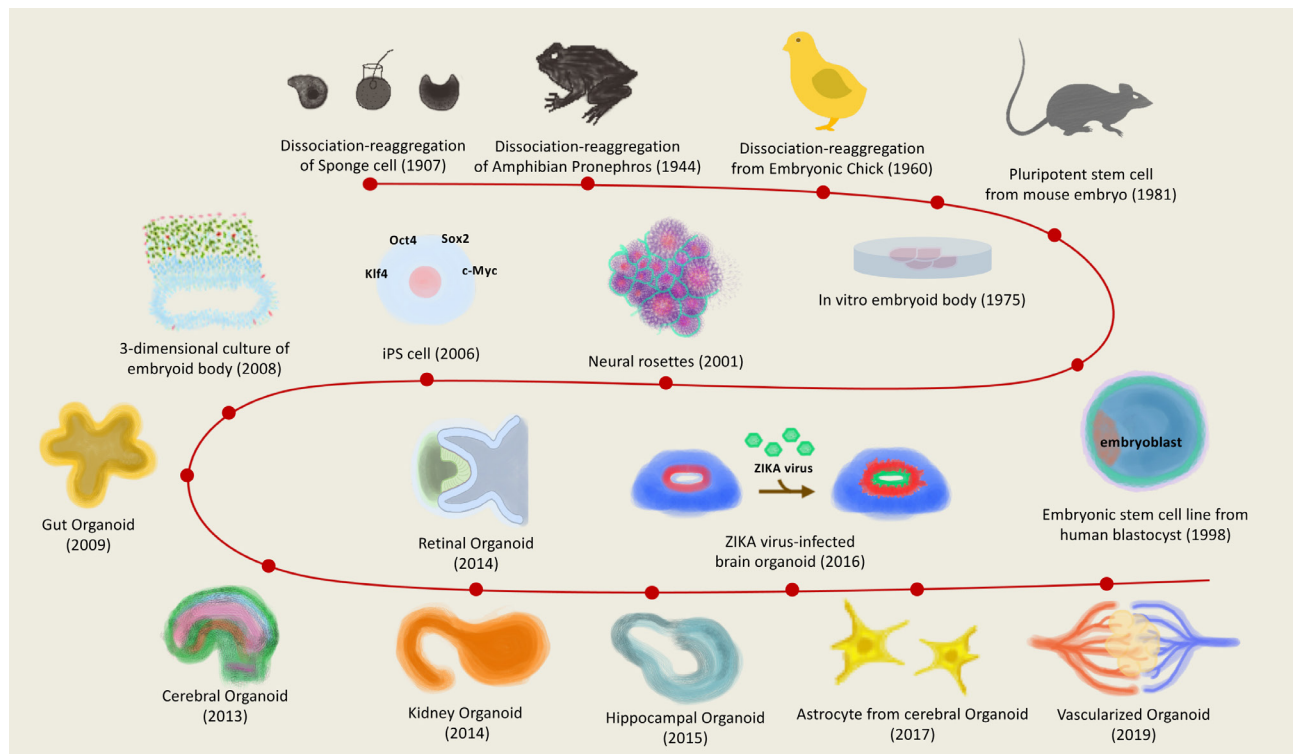


Fig. 1. History of brain organoid research. Organoid technology started from the research on re-aggregation of sponge cells, suggesting self-organization plays a key role in organ formation (Tung and Kü, 1944; Wilson, 1907; Zwilling, 1960). PSCs including embryonic stem cells and induced pluripotent cells were established (Martin, 1981; Takahashi and Yamanaka, 2006; Thomson et al., 1998). With these stem cell lines, various types of organoids are developed from intestine to hippocampus (Lancaster et al., 2013; Sakaguchi et al., 2015; Sato et al., 2009; Xia et al., 2014; Zhong et al., 2014). Brain organoid models have been widely used to study human brain disorders, including ZIKV infection. Bioengineering technologies are being developed to address the current limitations of organoids such as oxygen deprivation during long-term culture (Grebenyuk and Ranga, 2019).

tonomous cell sorting of the specific types of cells, leading to the formation of cell clusters and layers. It frequently occurs during re-aggregation of dissociated sponge cell, amphibian pronephros, and embryonic chick (Tung and Kü, 1944; Wilson, 1907; Zwillig, 1960). In addition, spatially restricted cell-fate decisions of daughter cells from progenitors also contribute to self-organization (Lancaster and Knoblich, 2014). Cell-cell adhesion works together with contractile cytoskeletons to generate intrinsic tissue-scale tension, which contributes to the curvature and shape of the tissue (Charras and Yap, 2018).

Based on self-organization, experimental culture systems have been developed to mimic multicellular organization of various tissues *in vitro* such as intestine (Sato et al., 2009), kidney (Xia et al., 2014), and retina (Zhong et al., 2014) (summarized in Fig. 1). The embryoid body is a multicellular aggregate derived from pluripotent stem cell, having a number of characteristics similar to the inner cell mass at the pre-gastrulation stage. The adherent culture of the embryoid body can generate a group of polarized neural progenitor cells (NPCs), called neural rosettes (Zhang et al., 2001). Neural rosettes resemble the early neural tube, with a preserved apical-basal polarity and the cleavage pattern of NPCs.

Moreover, neural rosettes recapitulate *in vitro*, the major milestones of cortical development, leading to the sequential generation of a diverse repertoire of neurons in proper temporal order (Gaspard et al., 2008; Shi et al., 2012; Yoon et al., 2014).

Based on these studies, 3D culture methods were developed for an embryoid body to mimic the developing mouse cortex (Eiraku et al., 2008) and human retinal tissue (Eiraku et al., 2011). Later, a protocol for the growth of human cerebral organoids was established by implanting an embryoid body in a Matrigel matrix to assist tissue formation, and by using a spinning bioreactor to increase gas and nutrient exchange (Grebenyuk and Ranga, 2019; Lancaster et al., 2013). In this cerebral organoid, the major features of the developing cortex, such as apical-basal polarity, interkinetic nuclear migration, division modes of neural stem cells, and the pattern of neuronal migration are well maintained. Moreover, the cerebral organoid exhibits the enlarged OSVZ, which is the basal proliferative zone in primates but not in mice. In addition, neural subtypes constituting all six cortical layers were produced by long-term culture using an improved spinning mini-bioreactor (Qian et al., 2016).

Early organoid differentiation protocols largely depended

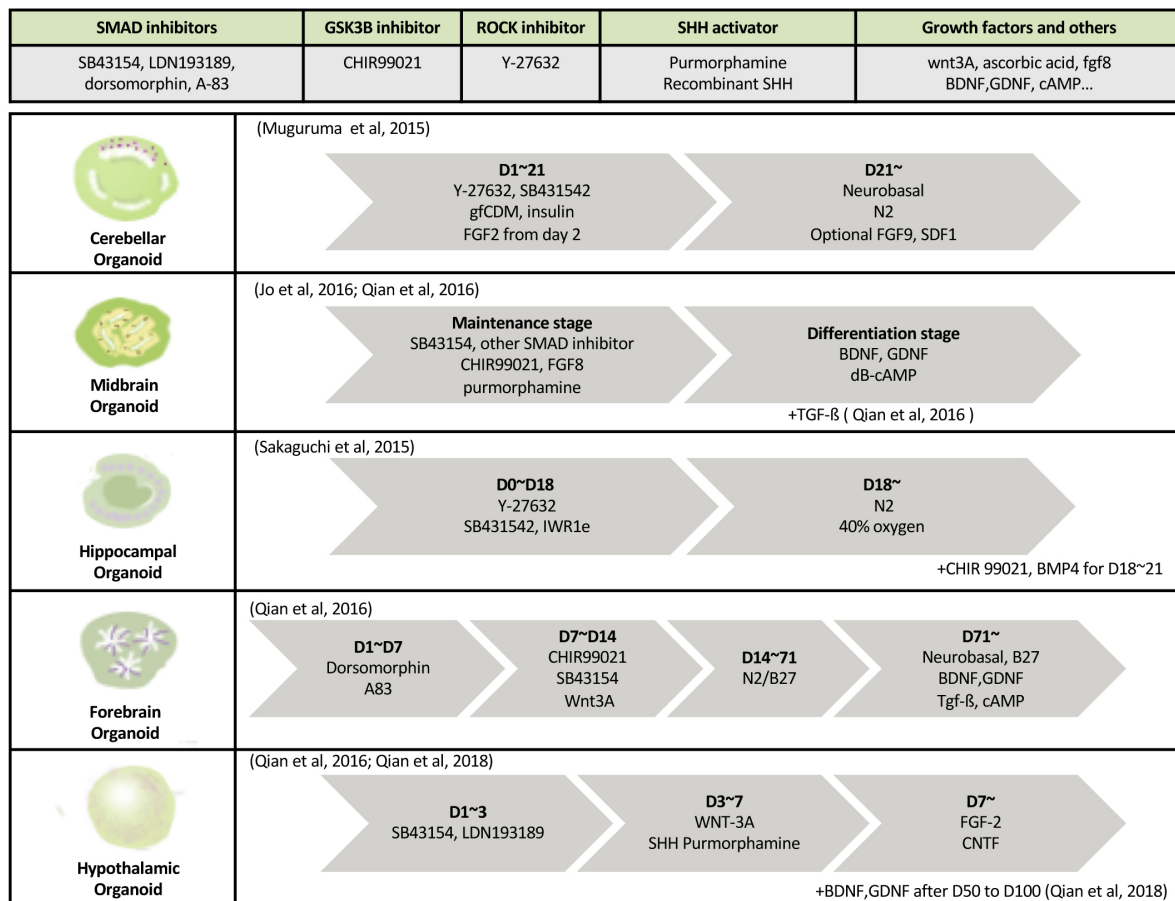


Fig. 2. Brief protocols of brain specific organoids generation. On the top, frequently used chemical reagents are summarized. On the bottom, brief schematics of organoid generation are described in time order, with critical components of culture media.

on the intrinsic signaling and self-assembly of stem cells. However, with region-specific differentiation factors, growth of other regions of the brain can be induced *in vitro* as organoids (summarized in Fig. 2). For example, a hindbrain neural tube-like structure that differentiates to form cerebellum-like organoids was generated by the sequential addition of FGF19 and SDF1 (Muguruma et al., 2015). Midbrain, hypothalamus, and hippocampus-like organoids can also be established by potent patterning cues, which initially instruct the differentiation of hPSC toward a homogeneous population of progenitors of specific brain regions (Jo et al., 2016; Qian et al., 2016; 2018; Sakaguchi et al., 2015). These brain region-specific organoids recapitulate the molecular, cellular, and structural features of the various areas of the human brain.

During the brain development, the guided cell migration driven by neurotrophic factors and cell-cell interaction is critical for properly assembly of the functional neural circuitry (Valiente and Marín, 2010). Excitatory neurons are generated from NPCs in the dorsal pallium and migrate radially to the

cortical plate. In contrast, GABAergic inhibitory neurons are generated from NPCs in the ventral subpallium and migrate tangentially to connect with excitatory neurons present in the cerebral cortex and modulate their activity (Kriegstein and Noctor, 2004). To model this process *in vitro*, organoids resembling the pallium and the subpallium were induced separately and then fused to form an assembloid. In assembloids, fluorescence-labeled inhibitory neurons successfully migrate tangentially, from the subpallium-like part to the pallium-like part (Grebenyuk and Ranga, 2019).

It is also feasible to produce mature and functional glia cells using organoid culture. Astrocytes present in cortical spheroids have well-functioning properties due to being tightly linked with neurons to form tripartite synapses (Pasca et al., 2015; Sloan et al., 2017). An oligocortical spheroid system was established that builds myelinated and functionally mature oligodendrocytes by using oligodendrocyte differentiation inducers and promyelinating drugs (Madhavan et al., 2018; Marton et al., 2019).

A highly coordinated gene expression program precisely

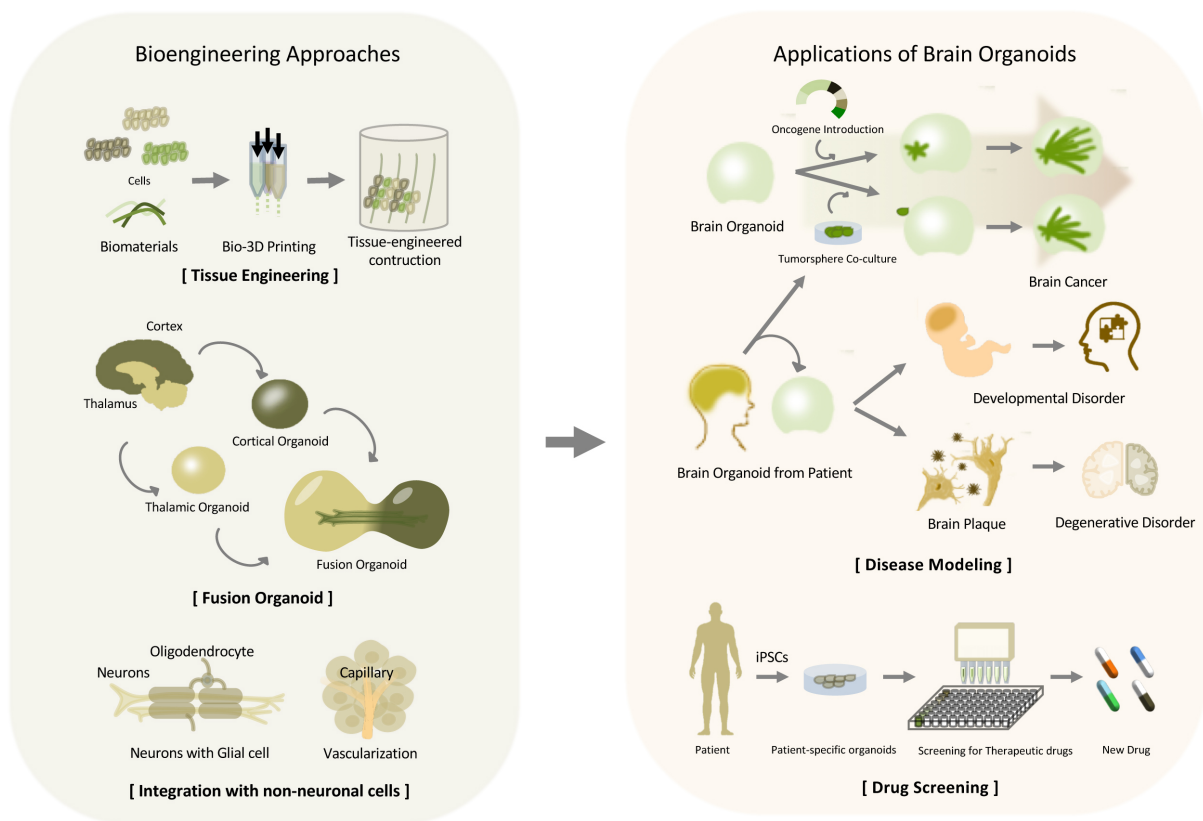


Fig. 3. Advances in brain organoid technology. Multidisciplinary bioengineering techniques provide a breakthrough to overcome the limitations of the current brain organoid technology. Tissue engineering approaches, such as 3D bioprinting, enable the rationally-designed organization of various type of cells in brain organoids. The region-specific brain organoids can be fused together to induce the integration of neural networks between distinct regions. Non-neuronal cells can be incorporated into brain organoids to model the interactions among neurons, glia, immune cells, and vasculature (left panel). The brain organoid technology has great translational potential for modeling brain disorders and screening therapeutic drugs. Brain organoids from patient-derived iPSCs are relevant platforms for studying brain cancer, neurodevelopmental and neurodegenerative disorders. Patient-specific mutations or oncogenes can be introduced into brain organoids using CRISPR/Cas9 technology to investigate the genetic mechanisms of brain disorders. In addition, a personalized drug screening using patient-derived organoids could predict drug efficacy before a drug treatment of patients (right panel).

orchestrates the spatiotemporal dynamics of the mammalian brain development. While structural and cellular features have been heavily studied, the intrinsic developmental program that regulates the stage and cell-type specific gene expression during human brain development is relatively unclear. The brain organoid system is a highly accessible and genetically modifiable model by which to study the gene expression program during human brain development. Indeed, comprehensive transcriptome comparisons between forebrain organoids and the human fetal cortex at different stages showed that organoid development is reminiscent of fetal human brain development at the transcriptome level (Madhavan et al., 2018; Marton et al., 2019; Qian et al., 2016). Moreover, the profiles of the N⁶-methyladenosine (m⁶A) modification on mRNA from forebrain organoids and the human fetal cortex showed significant overlap, suggesting that the epitranscriptome landscape during human brain development is well recapitulated by brain organoids (Yoon et al., 2017a). Single-cell RNA sequencing (scRNA-seq) has also been widely used to track the sequential changes of the transcriptome in individual cells and to identify the cellular composition at each time point of the organoid differentiation (Camp et al., 2015; Choi and Kim, 2019; Quadrato et al., 2017; Velasco et al., 2019). For example, analysis of the transcriptome of 31 different organoids using droplet-based scRNA-seq showed that brain organoids generated a broad diversity of cells, which are related to endogenous classes, including cells from the cerebral cortex and the retina (Quadrato et al., 2017; Velasco et al., 2019). In the future, single-cell level transcriptome analysis using various brain-region specific organoids will provide a deeper understanding of the regulatory mechanisms of the gene expression processes during the human brain development.

APPLICATIONS OF BRAIN ORGANOID SYSTEMS TO INVESTIGATE BRAIN DISORDERS

Over the last few years, the brain organoid system has been widely used to investigate human brain disorders (Fig. 3). Because brain organoids go through developmental steps similar to those of a human fetal brain, they are suitable to model neurodevelopmental disorders with actual pathogeny. In addition, brain organoids can contribute to fill the gap between the result from animal models and human patients of neurodegenerative disorders. Last, brain organoids have also modeled the etiology and the progression of brain cancer, which can be combined with genetic engineering and anti-cancer drug screening. In the near future, brain organoids are expected to provide a standard experimental model system for brain disorders.

Neurodevelopmental disorders

Neurodevelopmental disorders are the diseases that impair brain functions such as emotions, learning, sociality, or self-control due to perturbations in the developmental processes. Microcephaly, epilepsy, and intellectual disability are well known examples of neurodevelopmental disorders. In addition, psychiatric disorders, such as autism spectrum disease (ASD), schizophrenia (SZ), and bipolar disorder are also

results of abnormalities in developmental processes (Levitt and Veenstra-VanderWeele, 2015). According to statistical data, about 5% of the world population is suffering from neurodevelopmental disorders (Mitchell, 2011). Thus, it is important to understand the causes of neurodevelopmental disorders and to establish a proper preclinical model system by which to develop new treatments. Considering previously mentioned limitations of conventional model systems, brain organoids are expected to provide new breakthroughs in the study of neurodevelopmental disorders.

Microcephaly is one early example of a brain disease modeled using brain organoids. Microcephaly is characterized by a smaller head circumference, intellectual disability and seizures. Microcephalic cerebral organoids were first derived from iPSCs of a microcephaly patient who has a mutation in CDK5 regulatory subunit-associated protein 2 (*CDK5RAP2*), which is known as a genetic risk factor of microcephaly (Lancaster et al., 2013). The NPCs exhibited the reduced proliferation and premature differentiation in the patient-derived organoids compared to the control organoids. Moreover, the knockdown of *CDK5RAP2* in the control organoids led to similar phenotypes, suggesting that the loss-of-function in *CDK5RAP2* is a causative reason for microcephaly-associated cellular abnormalities in human cerebral organoids. This study was the first example in which a neurodevelopmental disorder was modeled using brain organoids, and suggests that patient-derived organoids, together with brain organoids gene-edited by CRISPR/Cas9 technology, will be largely helpful for understanding the genetic mechanisms of brain disorders.

In addition, brain organoids have also been used successfully to demonstrate the pathogenic pathways of infectious disorders such as Zika virus (ZIKV). Microcephaly can be caused by environmental factors that can affect fetal brain development during pregnancy. A ZIKV outbreak in South America and its suspected link with microcephaly led the World Health Organization to declare a global health emergency (Heymann et al., 2016). Despite the clinical evidence showing the ZIKV existed in the fetus and amniotic fluid of the infected mother (Calvet et al., 2016; Mlakar et al., 2016), a mechanistic understanding of how ZIKV induced damage during embryonic brain development was limited due to the variable quality and genetic background of the clinical samples. Therefore, human PSC-derived brain organoid models were adopted to study the cellular tropism and pathogenesis of ZIKV under controlled conditions. The developing human cortex is composed of different NPCs with unique properties, such as vRGCs, oRGCs, and intermediate progenitor cells (IPCs), which can be modeled by 3D brain organoids (Lui et al., 2011). In a human forebrain organoid, ZIKV exhibits tropism towards vRGCs and oRGCs over IPCs or immature neurons in human iPSC-derived brain organoids. Infected NPCs become viral factories, producing more infectious viral particles, leading to the propagation of ZIKV-infected cells throughout the brain organoids (Garcez et al., 2016; Qian et al., 2016). Meanwhile, intrinsic differences in the pathogenicity of different ZIKV strains have been extensively investigated using brain organoid models (African, Asian, American strains) (Cugola et al., 2016; Gabriel et al., 2017; Yuan et

al., 2017). On the other hand, host responses toward ZIKV were discovered by genome-wide transcriptome analysis on a ZIKV-infected brain organoid, which led to identifying the essential cell signaling pathway for viral infection (Dang et al., 2016; Watanabe et al., 2017). Moreover, whole-genome analysis of the methylome in a ZIKV-infected brain organoid showed that viral infection changes the host methylome of genes related to brain disorders, resulting in a long-term effect on the neurodevelopment of an infected newborn after delivery (Janssens et al., 2018). In addition, the brain organoid model can be also used as a platform for identifying interactions between viral proteins and host components (Yoon et al., 2017b), or screening noble drugs or signaling pathways that alleviate viral infection (Xu et al., 2016; Zhou et al., 2017).

As in the case of ZIKV infection, it is possible for a brain organoid to mimic the changes that occur in a fetal brain when it is exposed to a harsh environment. In a recent study, a human cortical spheroid (hCS) was used as an experimental model for hypoxic encephalopathy of prematurity; that is, reduction in cortical volume due to prenatal hypoxia (Pasca et al., 2019). *Tbr2*⁺ NPCs were reduced and prematurely differentiated in hypoxic hCSs, which can be rescued by inhibition of the unfolded protein response pathway. These examples highlight the potential for the use of human brain organoids to investigate the etiology of neurodevelopmental disorders related to genetic causes and environmental insult.

Brain organoid models are actively used to study mental disorders of which the etiology originates from neurodevelopment. For example, brain organoids derived from idiopathic ASD patients exhibited an accelerated cell cycle and overproduction of GABAergic inhibitory neurons (Mariani et al., 2015). Through genome-wide transcriptome analysis and gene network analysis, it was discovered that the *FOXG1* gene was coherently overexpressed in patient-derived brain organoids, which caused the excessive formation of inhibitory neuron. This was further verified by a *FOXG1* knockdown experiment. Another transcriptome analysis using cerebral organoids with *CHD8* haplosufficiency revealed a subset of dysregulated genes overlapping those of the idiopathic ASD organoids (Wang et al., 2017). In addition, a forebrain organoid derived from iPSCs of a cohort of ASD patients who had macrocephaly showed ASD-associated changes in the maturational sequence of early neuron development. This involved temporal dysregulation of specific gene networks and morphological growth acceleration (e.g., premature neurite outgrowth) (Schafer et al., 2019).

Timothy syndrome is another good example that showing the feasibility of using brain organoids as biological simulators. Timothy syndrome is a neurodevelopmental disorder involving severe epilepsy. It had been known that mutation in the *CACNA1C* gene is responsible for Timothy syndrome by producing abnormal inhibitory neurons. To recapitulate the migration of inhibitory neurons observed in the fetal forebrain, organoids of the dorsal forebrain and the ventral forebrain were separately generated with iPSC derived from Timothy syndrome patients and assembled *in vitro*. Imaging of the fluorescence-labeled inhibitory neurons in these fused organoids showed that the patient-derived inhibitory neurons

had impaired tangential migration due to cell-autonomous defects (Birey et al., 2017). This study was the first example showing the application of a fused organoid system to investigate brain disorders with abnormalities in the interactions among distinct brain regions.

Neurodegenerative disorders

Because it has been repetitively shown that rodent models of Parkinson disease and Alzheimer disease (AD) cannot reproduce the same pathophysiology as in human patients (Dawson et al., 2010; Raslan and Kee, 2013), the brain organoid model is now considered a better alternative, especially to investigate the early-stages of disease progression. For example, brain organoids derived from Familial AD patients displayed Amyloid-beta ($A\beta$) deposition and hyperphosphorylation of the Tau protein, representative biomarkers of AD (Raja et al., 2016). Another example showed that brain organoids can be used to study patient-specific variants, combined with CRISPR/Cas9 genome editing. The E4 allele of *APOE* (*APOE4*) is the most significantly associated genetic risk factor for sporadic AD, which markedly increases AD risk relative to the *APOE3* allele. Isogenic *APOE4* brain organoids generated by gene editing from healthy iPSCs displayed increased $A\beta$ aggregates and hyperphosphorylation of Tau. Converting *APOE4* to *APOE3* was sufficient to attenuate multiple AD-related pathologies in the brain organoids (Lin et al., 2018).

On the other hand, a midbrain-like organoid model from hPSCs was developed to study the pathophysiology of Parkinson's disease, which has functionally mature midbrain dopaminergic neurons. Interestingly, the midbrain organoids from human PSCs, but not those from mouse ES cells contained neuromelanin-like granules similar to those isolated from human substantia nigra tissues, implying the unique advantages of a human brain organoid system (Jo et al., 2016). In another study, isogenic 3D midbrain organoids with a Parkinson's disease-associated *LRRK2* G2019S mutation were generated by genome editing to study the pathogenic mechanisms associated with the *LRRK2* mutation. Midbrain organoids with the *LRRK2* mutation can recapitulate the pathological hallmarks and gene expression profiles seen in patients with *LRRK2*-associated sporadic Parkinson's disease (Kim et al., 2019a). These results suggest that a brain organoid system could be utilized as a platform to study the 3D pathophysiology and to develop noble therapeutics for neurodegenerative disorders.

Brain cancer

The nature of mutagenesis and metastasis makes cancer intractable. To develop an effective anti-cancer treatment, it is crucial to have a model system that reflects the genetic background of patients as well as the 3D multicellular environment of a primary tumor tissue. In this regard, a patient-derived brain organoid would provide an accessible, scalable, and easily manipulable system to understand the progression and the resistance of cancer and to screen anti-cancer drugs with patient-derived samples.

It was reported that a tumor organoid system was established from patient-derived samples with glioblastoma. Such tumor organoids can mimic the growth pattern of cancer

cells and their microenvironment better than a canonical 2D culture can. In the tumor organoid model of glioblastoma, cancer stem cells located on the peripheral side of the tumor organoid displayed a high turnover rate whereas cancer stem cells located at the hypoxic core in an organoid displayed a quiescent and senescent states (Hubert et al., 2016). This result suggests that the 3D tumor organoid represents the cellular environment of an *in vivo* primary tumor, including regional heterogeneity.

Not only tumor organoids directly derived from patients, but diverse experimental approaches have been also applied to examine the pathogenesis of brain cancers. When glioblastoma cells were isolated from a patient and grafted into cerebral organoids, the characteristics of the parental glioblastoma, such as growth pattern or tumor microtubule structure, were still preserved in the engrafted tumor. In addition, the glioblastoma showed higher resistance toward a chemotherapeutic drug and ionizing radiation in the cerebral organoids compared to the 2D culture (Linkous et al., 2019). By manipulating oncogenes or tumor suppressors using CRISPR/Cas9 in cerebral organoids, tumorigenesis of glioblastoma was successfully initiated and monitored *in vitro*. After transplantation of the organoid-derived cells to immune-deficient mice, the transplanted cells displayed characteristics of cancer-like invasiveness and angiogenesis, while having the biomarkers of glioblastoma (Bian et al., 2018; Ogawa et al., 2018).

Another study used brain organoids to evaluate the anti-cancer effects of chemotherapy. Two anti-cancer drugs, temozolomide and doxorubicin, were tested on a 3D heterotypic glioblastoma brain sphere model. The model was generated by incorporating a patient-derived glioblastoma into brain organoids. Interestingly, neither treatment altered the number of normal neuronal cells, and they exerted anti-tumor effects by selective induction of apoptosis (Plummer et al., 2019). These examples highlight the future potential of cancer research using diverse types of 3D brain organoid models.

FUTURE PERSPECTIVE

With a history of less than a decade, brain organoid technology is still in its infancy. Current protocols for the generation of brain organoids can mimic the human fetal brain around the second trimester, in terms of cellular and molecular composition (Camp et al., 2015; Pasca et al., 2015; Qian et al., 2016). Because the brain organoid does not have a circulation system with blood vessels, it mostly depends on simple diffusion from the culture medium for its supply of gas and nutrients. When culturing occurs over a long period, a substantial number of cells in the organoids undergo apoptosis due to a deficiency of oxygen and nutrients. Because the human brain continues to develop for several years after birth, it is necessary to establish an improved circulation system for brain organoids for extended *in vitro* culture. Recently, a blood vessel organoid was successfully generated from human PSCs, containing endothelial cells and pericytes that self-assemble into capillary networks. Human blood vessel organoids can be transplanted into mice to form a stable, perfused vascular

tree, including arteries, arterioles and venules (Wimmer et al., 2019). In the future, human brain organoid technology could be combined together with a blood vessel organoid to establish a functional closed circulation system, to support long-term culture and to study neurovascular interactions. An alternative for long-term culture might be transplantation of human brain organoids into a mouse brain. Neuronal differentiation and gliogenesis proceeded further in transplanted brain organoids compare to *in vitro* cultured organoids, and the vascular structure composed of mouse endothelial cells was well-formed in the transplanted brain organoid (Mansour et al., 2018).

The human brain is composed of various types of non-neural cells, not only neural cells derived from the neuroectoderm. Most of the current protocols induce the neuroectodermal fate first in the embryoid body; thus, the non-neural cells such as microglia, endothelial cells, hematopoietic cells, and meninges are largely missing. Hence, the current brain organoid cannot appropriately model the brain functions or disorders mediated by interactions in non-neuronal cells or interactions between non-neural cells and neural cells. One study reported that brain organoids generated without dual-SMAD inhibition innately contain mesodermal progenitors, which are able to differentiate into mature microglia instructed by the CNS microenvironment provided by neuroectodermal cells (Ormel et al., 2018). On the other hand, a co-culture system of 2D-differentiated microglia and a brain organoid could also be useful to investigate interactions between microglia and neural cells (Lin et al., 2018).

It has been proposed that the gyrification of the human cerebrum is critical to having large numbers of neurons in a small volume, leading to the evolution of higher cognitive ability. However, the gyrus and the sulcus formed by gyrification are not typically observed in brain organoids with the current protocols. Interestingly, a recent study described gyrification in brain organoids through the activation of the PTEN-AKT pathway (Li et al., 2017). This suggests that further technical advances could enable the generation of a larger brain organoids with a unique pattern of gyrification similar to that of an actual human brain. Because the frequently used rodent models (e.g., mouse models) do not have a gyrification process, our understanding of the cell biological and evolutionary basis of gyrification could be expanded by using brain organoid models. For example, a brain organoid on a chip grown from human ES cells provided a good platform from which to investigate the mechanical and physical properties of the human brain folding (Karzbrun et al., 2018).

In addition, consistency and reproducibility in cell types generated in brain organoid should be considered carefully. Batch variations among individual brain organoids such as differential composition of cell types could greatly hamper the correct interpretation of experimental results. A study highlighted that multiple hCSs showed similar cell-type composition and proportions by using single cell RNA-seq (Yoon et al., 2019). In addition, single-cell RNA-seq analysis revealed that dorsal forebrain organoids derived from different stem cell lines showed consistent cell-type composition after three months or six months. The developmental trajectories of cells in different batches of organoids were also consistent and

reproducible, and dorsal organoids had transcriptomic profile similar to that of a human fetal cortex (Velasco et al., 2019). These results suggest that the brain organoid is a tractable system for studying human brain development in a careful experimental setting.

The current protocols for brain organoids mostly rely on the self-organization capability of neuroepithelial cells. With the protocols that start by forming embryoid bodies, the initial experimental conditions such as the exact number of cells in an embryoid body, are poorly controlled. Because the undefined initial conditions and environmental factors during long-term culture result in high variability between organoid samples, it is technically very challenging to perform fine quantitative studies and large-scale unbiased screening. Bioengineering techniques are expected to provide a breakthrough approach to tackle these challenges in organoid systems. For example, by 3D bioprinting technology, prepatterned progenitors could be assembled into a single 3D structure to precisely define the initial culture material (Murphy and Atala, 2014; Vijayavenkataraman et al., 2018). The methods by which to engineer the initial organoid size, shape, and composition using microwell arrays, droplet-based microfluidics, and chemically programmed tissue assembly have been also actively developed (Karzbrun et al., 2019). By mixing extracellular matrix, signaling molecule, and other additives with the biomaterial, it is possible to provide a culture condition that is more similar to that during actual human brain development (Yin et al., 2016). In addition, microfluidic organ-on-a-chip technology can provide more controllable, and reproducible platform by assembling essential components of brain organoids into a small mechanical device (Park et al., 2019). However, high-throughput screening of new therapeutics using brain organoids is still challenging due to the long-term, and complex nature of the culture procedures. Nonetheless, mini-kidney organoids have been manufactured and analyzed in such manner using an automated robotic pipe line (Czerniecki et al., 2018). This suggests that advanced robotics and computerized phenotyping will contribute significantly to the enhancement of brain organoid technology compatible with high-throughput screening. Overall, combining a better understanding of the human brain development together with future bioengineering approaches will provide more advanced methods for generation of brain organoids.

The establishment of patient-derived models faithfully reproducing normal physiology and disease pathogenesis are essential for investigating molecular mechanisms, identifying new diagnostic and prognostic biomarkers, and personalized patient treatments. Because brain organoids derived from individuals maintain the major characteristics of the developing brain with identical genetic information, the brain organoid system has enormous potential to pave the way for personalized medicine for brain disorders. Brain organoid bio-bank, a collection of various pathological types of patient-derived organoids, would greatly facilitate our understanding of brain disorders as well as support development of new therapeutics. Collections of various types of tumor organoids have already been established and are good examples that show the benefits of organoid bio-banks. Using these bio-banks, recent studies have examined tumor subtype heterogeneity

and performed large-scale therapeutic screenings to provide useful resources for studying both cancer cell biology and precision cancer therapy (Kopper et al., 2019; Sachs et al., 2018; van de Wetering et al., 2015; Yan et al., 2018). In the sense that brain tissue is usually hard to gather compared to tumor tissue, the brain organoid bio-banks would be an invaluable resource to reveal much information about individual heterogeneities, molecular pathogenesis of brain disorders, and potential drug targets.

Is it possible to make a neural network in a brain organoid with computational ability, memory, and intelligence? Surprisingly, spontaneous neural activity with periodic oscillatory events similar to those observed in the preterm human brain has been detected with an electroencephalogram in brain organoids (Trujillo et al., 2019). Additionally, there is a study in which photosensitive retinal neurons in a brain organoid were able to react to light stimuli (Quadrato et al., 2017). Further progress in brain organoid technology may allow innovation aimed at creating biological machines with functions similar to some in the human brain (Buchanan, 2018).

With less than a decade of history, brain organoid technology has already started to have major impacts on medical research. Brain organoids are expected to become invaluable models for better understanding of the fundamental biology of brain development and function and brain disorders.

Disclosure

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