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Modeling neurological disorders using brain organoids

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Abstract

Neurological disorders are challenging to study given the complexity and species-specific features of the organ system. Brain organoids are three dimensional structured aggregates of neural tissue that are generated by self-organization and differentiation from pluripotent stem cells under optimized culture conditions. These brain organoids exhibit similar features of structural organization and cell type diversity as the developing human brain, creating opportunities to recapitulate disease phenotypes that are not otherwise accessible. Here we review the initial attempt in the field to apply brain organoid models for the study of many different types of human disorders across a wide range of etiologies and pathophysiologies. Forthcoming advancements in both brain organoid technology as well as analytical methods have significant potential to advance the understanding of neurological disorders and to uncover opportunities for meaningful therapeutic intervention.

Introduction

The human nervous system is one of the most complex tissues in biology, particularly in terms of its cell type diversity, cellular architecture and organization, and functional connectivity. Diseases related to its development, degeneration, cancer, and exposure to environmental insults have been difficult to study due to the complexity of their unique

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pathophysiologies and difficulties in accessing human specimens. Animal models and human post-mortem analyses have been absolutely invaluable; however, many aspects of brain development and diseases exhibit essential human-specific features, and prospective studies remain nonetheless indispensable for mechanistic investigation and targeted interventions based on mechanistic understandings [1-3].

Rapid progress in stem cell technologies over the past decades, including reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) and differentiation of pluripotent stem cells, including both iPSCs and embryonic stem cells (ESCs), has widely enabled access to human neural cells within an *in vitro* system that permits extensive experimental manipulation for phenotypic and mechanistic studies [4, 5]. These efforts have recently culminated in the development of brain organoids, which are 3D structures that resemble the nervous system and can be used as experimental models for studying normal organogenesis and disease pathophysiology [6, 7]. With optimized culture conditions as well as a degree of intrinsic self-organization, brain organoids are able to capture certain aspects of the tissue architecture and cell type diversity of the human nervous system through various stages of development.

These features of brain organoids offer unique opportunities to model developmental processes and disease phenotypes not otherwise available with alternative approaches, such as 2D monolayer and neurosphere cultures [8], Certainly, brain organoids do not fully or perfectly recreate the developing human brain, and they also suffer from a number of technical limitations; however, existing methods have proven adequate to recapitulate important features of diseases and yield valuable insights, and forthcoming advancements, such as incorporation of other non-neural cell types and vasculature, are expected to greatly improve the fidelity and scope of this model system.

In this review, we focus on the use of brain organoids in modeling neurological diseases, highlighting the specific advantages of this model system as well as discussing areas for further development. Overall, brain organoids are a young and nascent field with demonstrated and substantial promise to deepen the understanding of basic neurobiology, to uncover specific and actionable insights into neurological diseases, and to advance the development of effective therapeutics.

Organoid models of early brain development

In less than a decade, brain organoids have emerged as novel and useful tools for modeling normal human neural development and neurological disorders in a 3D *in vitro* culture system. Brain organoids are generated from pluripotent stem cells (PSCs), and with recent advancements in stem cell technologies, species-specific – often human – as well as patient-specific models of brain development and diseases are readily accessible. The study of species differences and evolution lies outside the scope of this review and we focus on research efforts that use brain organoids from patient-derived iPSCs or genome-edited human PSCs to study disease processes with the appropriate genetic backgrounds.

Protocols to generate brain organoids can be broadly divided into unguided and guided methods [6]. Unguided methods forgo the use of targeted patterning factors and rely on intrinsic cues and self-organization capabilities of PSC aggregates under optimized growth conditions. Although this method generates an enormous diversity of cell types, encompassing many areas of the brain, as well as retina, choroid plexus, and mesoderm, the stochasticity and iPSC line-to-line and organoid-to-organoid variability remain major challenges with this approach [9, 10]. Guided methods target key signaling and morphogen pathways using small molecules and growth factors through at least the progenitor patterning stage to yield brain region-specific organoids. Protocols to model many brain regions, including the forebrain, midbrain, hypothalamus, hippocampus, and many others, have been reported [11-13]. As the brain organoid toolkit expands to encompass more individual and combinations of brain regions, selection of an appropriate model system will be important to best model the disorder or biological process of interest.

With both unguided and guided methods, brain organoids consist of a variety of cell types, including a number of different stem and progenitor cells, neurons, and glia, all of which arise in sequence, reflecting the temporal dynamics of the genesis of these cells in normal brain development [11, 14, 15]. Thus, the emergence and diversity of these cell types in brain organoids offer the opportunity to model phenotypes, such as proliferation and premature differentiation, as well as to examine the cell type-specific effects of various genetic mutations or environmental exposures.

Brain organoid exhibit similar transcriptome, epitranscriptome and epigenome as the developing brain [11, 14-16]. Furthermore, some of the human-specific features of organoids can be captured in the presence and abundance of certain cell types, such as outer radial glia (oRGs) [11], which are relatively few in rodents [17]. Finally, brain organoids are uniquely defined by their three-dimensional architecture, often containing defined laminar zones of progenitor cells and neurons they give rise to [18]. A number of disorders involve defects in cellular organization and tissue architecture, and brain organoids are well suited to model the underlying biological processes of these disorders. In all, the innate complexity of brain organoids offers opportunities to study cellular processes and molecular pathways that are essential to human diseases and yet not readily available in alternative *in vitro* model systems.

Organoid models for congenital developmental disorders with genetic etiologies

Structural abnormalities

One of the major advantages of brain organoids over existing 2D culture models as well as neurospheres is the presence of cellular organization and cytoarchitecture that are formed through self-organization and the application of certain extracellular matrix factors. Cerebral and forebrain organoids consist of neural rosette-like structures with a fluid-filled ventricle at the center surrounded by sequential layers of neural progenitor cells (NPCs) and neurons. These NPCs consist of various radial glia subtypes, and they demonstrate apical-basal polarity, display typified mitotic translocation and cleavage angles, and act as a scaffold for

neural migration. Thus, cortical organoids are uniquely useful for modeling diseases where structural defects are prominent features of the clinical phenotype (Table 1). Future advancements may further elaborate the structural characteristics of cortical organoids especially those that emerge in later stages of development, such as the six neuronal layers of the neocortex and prominent gyri and sulci along the cortical surface.

Microcephaly: Microcephaly is clinically defined as an occipitofrontal head circumference of more than 2-3 standard deviations below the mean for age and gender. Although the etiologies are diverse, most cases of prenatal onset microcephaly are thought to involve impaired NPC function and diminished overall neurogenesis. Brain organoids are important in modeling this disease process as mice lack the primate-enriched and highly neurogenic outer subventricular zone (oSVZ) where oRGs largely reside. This is thought to play a role in the more modest phenotypes observed in mouse models that bear genetic alterations [19, 20], whereas prominent phenotypes have been consistently observed in cerebral organoid models with causative pathogenic mutations in a number of different genes, such as a centrosomal protein, CDK5RAP2, and a mitotic spindle protein, ASPM [10, 21].

Studies in brain organoids have also identified a number of novel mechanisms and pointed towards potential therapeutic strategies. Centrosome dysfunction and cilium disassembly impairment were found in a cortical organoid model of Seckel syndrome, which is caused by mutations in a centromeric protein, CPAP, also known as CENPJ [22]. Cilium disassembly impairment and NPC depletion were also found in cerebral organoids with mutations in centrosomal protein WRD62 [20]. Mutations in CHMP1A can lead to microcephaly with pontocerebellar hypoplasia, and cerebral organoid models demonstrated that impaired Shh signaling can affect the maintenance of NPCs and pace of differentiation [23]. Aicardi-Goutières syndrome (AGS) can arise from mutations in TREX1 and is an inflammatory disorder with severe neurologic deficits and microcephaly. Cerebral organoid models of AGS demonstrated smaller sizes and increased apoptosis in neuronal regions, which were rescued upon treatment with reverse transcriptase inhibitors, Lamivudine and Stavudine [24].

Lissencephaly.—Lissencephaly is characterized by the lack of gyri and sulci on the cerebral cortex and is thought to be due to impaired neuronal migration. Although animal studies with genetically manipulated mice have been informative, their intrinsic lissencephaly places clear limitations on the fidelity of these models, and iPSC-derived brain organoids may bridge the gap to the human disease. Neuronal migration defects were found in cerebral organoids with pathogenic mutations in microtubule proteins KATNB1 and TUBA1A as well as a cerebral organoid model of Miller-Dieker syndrome (MDS) [25-27]. This MDS model also revealed a reduced NPC pool due to increased apoptosis and reduced vertical divisions, oRG-specific mitotic defects, and imbalanced NPC proliferation and differentiation [28].

The gyri and sulci in the human brain are largely formed in the third trimester; however, it remains difficult to model this later period in prenatal development using brain organoids [29]. Most brain organoids do not bear prominent gyrification, which may be due to a number of different challenges, such as inadequate organoid maturation; however, novel

approaches have been pursued to capture aspects of this essential feature of the human brain. PTEN mutation leads to NPC expansion and surface folding in brain organoids, which was only observed with human, but not mouse, cell lines [30]. Although non-physiologic, this mutant background may be useful in certain circumstances to study gyrification and model lissencephalies. Alternatively, a brain-organoid-on-a-chip approach was used to study the biomechanics of folding and identified contraction at the organoid core and expansion at the perimeter as opposing forces leading to wrinkling [31]. Brain organoid models with alterations in LIS1, which is located within the chromosome 17 deletion locus in MDS and is strongly associated with lissencephaly, demonstrated fewer wrinkles than isogenic controls and altered biomechanical properties.

Periventricular heterotopia.—Periventricular heterotopia (PH) is a condition where impaired neuronal migration leads to heterotopic neurons residing adjacent to the ventricles. Examination of *de novo* coding variants identified in patients with PH revealed a loss-of-function mutation in PLEKHG6, specifically transcript variant 4 (PLEKHG6_4), which is associated with oRGs and does not have an ortholog in mice [32]. Genetic manipulation of PLEKHG6_4 expression in cerebral organoids by electroporation led to altered neuronal migration, periventricular accumulation of neurons, and disruption of apical surface integrity that influenced the behavior of adjacent unmanipulated neurons in a non-cell-autonomous manner. Mutations in protocadherins DCHS1 and FAT4, often in one allele, are associated with PH, and cerebral organoids bearing these alterations demonstrate the stereotypical feature of ectopic neuronal clusters near the ventricle [33]. In contrast, mice, even with homozygous knockout of Dchs1 and Fat4, do not demonstrate abnormal cortical development [34]. This is similar in lissencephaly, a disease also with neuronal migration defects, where genetic mutations found in patients can yield modest or no relevant phenotypes in mouse models [35]. Thus, brain organoid models offer the opportunity for investigating underlying mechanisms of neuronal migration, especially those that may not be adequately recapitulated in other model systems.

Neuropsychiatric disorders

A number of neuropsychiatric disorders, including schizophrenia, autism spectrum disorder (ASD), and intellectual disability (ID) are thought to involve important pathophysiological processes during early brain development [36]. Although the clinical focus in these diseases is on the behavioral outcomes, brain organoids may serve as useful models to study the molecular and cellular alterations that occur in early developmental stages. These diseases have complex and poorly understood genetic and environmental etiologies, and cases with definitive and causative genetic alterations – though rare – have served as tractable starting points to build informative disease models.

Schizophrenia.—Schizophrenia is a severe mental disorder affecting numerous domains of psychological function, and although its onset is in early adulthood, a number of neurodevelopmental events remain closely linked with its pathogenesis. DISC1 is a scaffolding protein that interacts with many different pathways and its rare mutations have been genetically linked to a number of major psychiatric disorders, including schizophrenia [37]. Prior studies in mouse, zebrafish, and other cell culture models have demonstrated that

DISC1 mutations lead to dysregulated WNT signaling and neural progenitor activity [38, 39]. Brain organoids modeling a chromosomal translocation disrupting the DISC1 locus demonstrated morphological changes and reduced proliferation, which could be rescued with WNT pathway antagonism and phenocopied by WNT pathway agonism [40]. Another DISC1 brain organoid model with a rare 4 base-pair frameshift mutation found in major psychiatric disorder patients [41] revealed that disruption of the DISC1/NDEL1 complex leads to delayed cell-cycle progression and reduced proliferation, consistent with studies in mouse models and 2D cell culture models [42]. A sliced forebrain cortical organoid model was recently developed, which exhibits distinct upper and deep cortical layers with segregation of neuronal subtype markers [18]. In this model, the frameshift DISC1 mutation leads to deficits in cortical neuron subtype differentiation and failure of segregation of upper and deep cortical neuronal layers [18]. Notably, dysregulation of the laminar expression of neuronal subtype marker genes has been observed in brains from children with ASD [43]. Cerebral organoids with chromosome 16p13.11 microduplication demonstrated reduced proliferation as well as dysregulation of the NFκB p65 pathway, which could be restored using pharmacologic agents and genetic manipulations to rescue the proliferation phenotype [44]. Cerebral organoids from patients with schizophrenia but without any canonical alterations demonstrated aberrant cortical organization, which could be linked to nuclear FGFR1 signaling and phenocopied with pharmacologic inhibition of this pathway [45]. Overall, these models of schizophrenia have yielded insights into the cellular processes associated with disease-associated variants, especially into defects in cellular organization and morphogenesis that would not be well captured in other cell culture models. However, large domains of analyses, such as alterations in neuronal network connectivity, remain largely unexplored.

Autism Spectrum Disorder.—ASD is a neurodevelopmental disorder characterized by defects in social communication and social behaviors. Multiple lines of evidence in mouse models and human patients have implicated intemeurons in the pathogenesis of this disease [46], and brain organoid models from different genetic backgrounds have been consistent with this prior knowledge and expanded upon them. For example, brain organoid models of idiopathic ASD exhibited increased production of GABAergic neurons, which was in part attributable to increased expression of FOXG1 [47]. This study highlights an advantage of brain organoids as mouse and other cell culture models would not be suited to examine phenotypes involving a number of different cell types within an undetermined causal genetic background. Cerebral organoids with mutations in CHD8, a chromatin remodeler and a ASD risk gene, showed dysregulated expression of genes important in GABAergic neuron differentiation, consistent with results from the study of idiopathic cases [48]. A novel model of Timothy syndrome, a disease that shares socialization and communication phenotypes with ASD, bearing mutations in CACNA1C was developed by fusing cortical and subpallium spheroids into an assembloid, which then demonstrated impaired intemeuron saltation and mobility that could be rescued upon treatment with L-type calcium channel inhibitors [49]. Future studies examining the network integration of these intemeurons and resulting excitation-inhibition balance may build on this body of work.

Intellectual disability.—Intellectual disability is a neurodevelopmental disorder with deficits across a broad spectrum of intellectual function. Down Syndrome (DS) is caused by amplification of chromosome 21 leading to developmental and intellectual disabilities. OLIG1 and OLIG2 are located on chromosome 21 and have been previously implicated in DS [50-52]; however, the expression patterns of these two transcription factors differ between mouse and human, where in humans OLIG2, but not OLIG1, is abundantly expressed in populations of neural progenitors and is thought to play a more important role in DS [53-56]. Brain organoid models of DS showed overproduction of OLIG2-expressing ventral forebrain progenitors and GABAergic interneurons [57]. Injection of dissociated organoid cells into neonatal mice led to increased GABAergic neurons and behavioral deficits, both of which could be reversed with knockdown of OLIG2, thus better defining the link between OLIG1/2, interneuron development, and DS related phenotypes. Organoid models of Rett syndrome demonstrated impaired neuronal differentiation, which could be recapitulated with MECP2 knockdown and were linked to increased levels of miR-199 and miR-214, two miRNAs that impair neuronal differentiation by altering ERK and AKT signaling [58].

Other diseases

Gangliosidosis.—Gangliosidosis is a type of lysosomal storage disorder where gangliosides accumulate aberrantly due to inborn errors of metabolism. Organoid models of GM1 and GM2 gangliosidosis demonstrate accumulation of these lipids, which can lead to cerebral organoid degeneration and be rescued with targeted gene therapy [59, 60].

Pelizaeus-Merzbacher disease.—Pelizaeus-Merzbacher disease (PMD) is an X-liked monogenic leukodystrophy caused by a mutation in PLP1, leading to defects in myelin production. Oligocortical spheroids were developed by supplementation with oligodendrocyte lineage promoting factors, and models of PMD showed phenotypes of perinuclear accumulation of PLP1 and reduced oligodendrocytes, which could be rescued with PERK inhibitors [61].

Organoid models for congenital diseases with environmental exposures

Viral infection

A number of infectious diseases have prominent neural and neurodevelopment sequelae that can lead to lasting deficits, and brain organoids have played an important role in studying these diseases, particularly in Zika virus infection (Table 2). One area of particular advantage is the cellular diversity within brain organoids, which offers unique opportunities to examine viral tropism as well as to study cell type-specific responses to these exposures. As brain organoids lack immune cells, and therefore are not suitable for understanding pathogen-host immune responses. Mouse models of viral infections are advantageous in this aspect, but lacking species specificity of essential viral functions [62, 63].

Zika virus.—Zika virus is a flavivirus that is primarily transmitted by mosquitos, and although the symptoms of infection in adults are mild, prenatal exposure and infection have been linked to severe neurodevelopmental defects, including microcephaly. Since the 2015

outbreak of Zika Virus in Central and South America, brain organoids have been used by numerous research groups as a leading model system to identify cellular tropism, mechanisms of infection, and potential therapeutic options [64]. This body of research highlights how leveraging the unique features of brain organoids, such as its cell type diversity and structural organization, can lead to important insights in domains ranging from basic disease biology to drug discovery [65, 66].

Early studies using brain organoid models of Zika virus infection were the first to reveal viral tropism for NPCs, leading to reduced overall size and numbers of NPCs and neurons [11, 67, 68]. AXL was proposed as a candidate viral entry receptor given its enriched expression in radial glia cells, however, subsequent studies in AXL knockout brain organoids showed largely unchanged susceptibility to Zika virus infection, suggesting that AXL is not essential for viral entry [69, 70]. Co-culture models of iPSC-derived microglia-like cells with neural spheroids and human primary monocytes with brain organoids demonstrated that these non-neural cells may be suitable viral carriers and mediate dissemination into the developing brain [71, 72]. Zika virus infection of NPCs leads to reduced proliferation and premature differentiation, and is also characterized by altered division planes, impaired centrosome function, disrupted apical adherens junctions, disorganized radial glia morphology, and activation of TLR3 signaling pathways [73-75]. NS2A protein encoded by the Zika virus genome was found to disrupt adhereins junctions of radial glia in forebrain organoids, resulting in reduced proliferation and premature differentiation [76]. Furthermore, Zika virus infected brain organoids showed differential DNA methylation at loci associated with neurodevelopmental and psychiatric disorders, suggesting the possibility of long-term consequences [77]. Finally, a number of drug screens followed with validation in brain organoids have identified compounds such as emricasan, hippeastrine, 25-hydroxycholesterol and certain antibiotics and antivirals as promising candidates for treating Zika virus infection [78-83].

Japanese encephalitis virus (JEV).—Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus that is the leading cause of viral encephalitis in Asia, and although symptomatic infection is uncommon, neurologic sequelae can be severe and long lasting. Cortical organoids models of JEV revealed viral tropism for astrocytes and NPCs, especially oRGs, leading to reduced organoid size and increased cell death [84]. Interestingly, older organoids exhibited less susceptibility to JEV infection as well as greater activation of interferon signaling pathways.

Cytomegalovirus (CMV).—CMV is a ubiquitous pathogen, and prenatal infection can lead to neurodevelopmental defects, including microcephaly; however a major challenge of studying CMV is its strict species specificity [85]. Brain organoid models of CMV infection demonstrated microcephaly, disrupted cellular organization, and abnormal calcium signaling, which could be prevented with neutralizing antibodies or partially rescued with maribavir, an experimental antiviral agent [86, 87].

Brain organoid modeling has led to significant novel insights into viral infection of the developing nervous system, especially with respect to cell type tropism, morphological defects, and potential novel therapies. Future incorporation of additional cell types, such as

microglia and lymphoid cells, into the brain organoid model in a biologically faithful manner will help to better understand complex host-pathogen interactions and their consequences.

Chemical agents and radiation

The developing brain is often susceptible and sensitive to the exposure or depletion of many different non-biological agents, including oxygen, drugs of abuse, pharmacological agents, frank toxins, and radiation. As with infectious diseases, modeling these non-biological exposures in brain organoids presents an important advantage of studying differential susceptibly and responses in a system where all these different cell types are integrated in a biologically meaningful manner. These published research studies lay the groundwork for future opportunities to screen for developmental neuro-teratogenicity across broad drug development and environmental applications (Table 2).

Hypoxia.—Prenatal hypoxic injury is a common cause of neurodevelopmental deficits as even transient episodes can cause cellular damage that lead to long term neuropsychiatric sequelae. Prior studies in mouse models identified SOX2-expressing cells at the basal SVZ that form a proposed oSVZ-like cell layer and are sensitive to oxygen levels during development [88]. However, given the lack of a true oSVZ in mice and species-related differences in cortical progenitor pools, the implications of these findings to human pathophysiology remain unclear. Brain organoid models of hypoxia show reduced numbers of progenitor cells, including oRGs and intermediate progenitors, as well as substantial transcriptomic alterations, which can be rescued upon reoxygenation, treatment with an integrated stress response inhibitor, ISRIB, or treatment with minocycline [89-91]. Endothelial cells and other elements of cerebrovascular biology play an essential role in hypoxic injury, and brain organoids that incorporate these components may offer a more complete model system in studying this disease process in the future.

Drugs of abuse: alcohol, cocaine, nicotine, and 5-MeO-DMT.—Prenatal exposure to drugs of abuse is widely prevalent and can lead to long lasting changes in brain development. Exposure of brain organoids to ethanol, cocaine, and nicotine yielded common phenotypes of premature neuronal differentiation and abnormal neurite outgrowth [92-94]. Notably, exposure to cocaine, a stimulant that inhibits the reuptake of monoamine neurotransmitters, also led to CYP3A5 induced production of reactive oxygen species. 5-MeO-DMT is a serotonin-like molecule with psychoactive and hallucinogenic properties, and exposure in brain organoids lead to dysregulation of proteins associated with inflammation, long term potentiation, and cytoskeletal dynamics [95]. Many of these drugs of abuse have neuromodulatory activity, and studying these effects on the different neuronal populations at different degrees of maturation may yield insights to how early exposure lead to the observed clinical behavioral phenotypes.

Pharmacologic agents.—The large majority of pharmaceutical drugs have an unknown risk in pregnancy despite their necessity and widespread use in pregnant women [96]. Mouse models are enormously useful but also complicated by host metabolism, maternal-fetal biology, and species differences in key developmental programs [97, 98]. The impact of

these challenges is exemplified by the antiemetic, thalidomide, which yields almost no teratogenicity in mice but severe deformities in humans [99, 100]. In cerebral organoid models, vincristine, a microtubule destabilizing anti-cancer agent, and tranylcypromine, a monoamine oxidase inhibitor, both yielded reduced neural and glial cell numbers [101, 102]. Further genomic analyses found that vincristine exposure dysregulates extracellular matrix associated genes, whereas tranylcypromine exposure increases H3K4 dimethylation. In contrast, exposure to imidazole antifungals leads to the generation of oligodendrocytes and remyelination through the inhibition of CYP51 and the subsequent accumulation of lanosterol [103]. Although these case studies of individual drugs demonstrate the potential of brain organoids, scaling to mid- or high-throughput screens for teratogenicity will require high volume sample processing pipelines as well as suitable phenotypic standards for identifying toxicity.

Toxins and radiation.—Toxin and radiation exposures are ubiquitous in the environment, and brain organoids can be useful in understanding their toxicity, mechanisms, and neurologic sequelae. Gamma irradiation of cerebral organoids leads to a thinned neuroepithelium and reduced metabolic output that could be rescued by pretreatment with rapamycin or subsequent treatment with minocycline [104]. Bisphenol A is a component in many plastics and its exposure in forebrain organoids at very high doses leads to reduced neural progenitor cell proliferation and thinned ventricular zone thickness [11]. Expanding this to larger scale, a screen of compounds known to be toxic and non-toxic in brain organoids was analyzed by RNA sequencing and used to generate a linear support vector classifier that had a >90% classification accuracy [105].

Organoid models for neurodegenerative diseases

Although brain organoids are more often designed and thought of as models of brain development, their application towards studying neurodegeneration have yielded models that recapitulate certain essential phenotypes, such as the presence of misfolded protein aggregates (Table 3). Notably, secreted proteins and extracellular deposits may accumulate to higher levels in the interstitial spaces in this model system simply due to the 3D aggregation of these cells even without the typified structure of brain organoids [106]. With the growing appreciation of the prion-like behavior observed in these diseases, studying neuronal diversity and connectivity may contribute to understanding how these pathologies may spread and disseminate. Neuronal and glial maturation as well as the potential interference of progenitor cells are concerns for the validity of brain organoids as models of neurodegenerative diseases, and they represent areas for further investigation and development.

Alzheimer’s disease.

Alzheimer’s disease (AD) is an age-related neurodegenerative disease characterized by neuronal cell loss, amyloid aggregation, and hyperphosphorylated tau. Transgenic mouse models exist for AD that mimic a range of pathologies [107, 108], however, a number of species-specific differences, such as the expression of tau isoforms [109, 110], and failed clinical trials for drugs that showed promising results in mice have prompted the

development of human brain organoid models that also have the added benefit of adaptability to high throughput drug screening [111-113]. Brain organoid models of familial AD with mutations in APP or PSN1 yield amyloid aggregates, hyperphosphorylated tau, endosome abnormalities, and increased inflammatory markers, which could in part be rescued with β- and γ-secretase inhibitors [114-116]. Models of sporadic AD showed that APOE4 brain organoids exhibit increased Aβ puncta and phosphorylated tau, as well as upregulation of neurogenic genes [117, 118]. Beyond genetic models of AD, treatment of wild-type brain organoids with amyloidogenic Aftin-5 led to increased levels of Aβ42 and Aβ oligomers [119]. These brain organoid models of AD capture essential biochemical features of the disease, however, the lack of cellular components such as microglia and vasculature, as well as the relatively low synaptic activity and cellular immaturity are important challenges that will require ongoing evaluation and innovation [120].

Frontotemporal dementia.

Frontotemporal dementia is a common type of early-onset neurodegenerative disease with behavioral, language, and motor impairments. Mechanistic studies in brain organoids validated an important mechanistic association between p25, a disease-related fragment of p35 that enhances CDK5 activity, and tau phosphorylation in a human system that had been previously observed in mouse models [121-123].

Huntington's disease.

Huntington's disease (HD) is an inherited neurodegenerative disease caused by excessive CAG repeats in the huntingtin gene. Brain organoid models demonstrated poorer neuroecoderm patterning with increasing CAG expansion, disrupted cortical differentiation and organoid cytoarchitecture, which could be partially rescued with knockdown of HTT and pharmacologic inhibition of ADAM10 [124].

Creutzfeldt-Jakob disease.

Creutzfeldt-Jakob disease (CJD) is a rare, fatal, and fast-progressing neurodegenerative disease that is caused by abnormal forms of prion protein. Wild-type cerebral organoids were exposed to brain homogenates from two patients with sporadic CJD, leading to prion protein uptake and propagation [125].

Hereditary Spastic Paraplegia.

Hereditary spastic paraplegia is characterized by progressive degeneration of the corticospinal tracts. Cerebral organoid models with SPG11 mutations demonstrated premature differentiation, reduced overall proliferation, and larger ventricles, which could be partially rescued with GSKβ inhibitors [126].

Organoid models for cancer

Organoid models of cancer have emerged across a number of different tumor types and organ systems to better preserve the cellular heterogeneity and microenvironment in an *in vitro* system (Table 4). Some brain tumor organoid models are similar to brain organoids in that they are generated from PSCs with defined genetic alterations, however, other models

are derived from primary surgical tissue and contain patient-specific genetic features. Notably, the patient-derived organoids contain many different types of non-neoplastic cells, including immune cells, creating opportunities to study these intercellular interactions and how they might influence tumor growth or response to treatment. These emerging models offer unique opportunities to study tumorigenesis, cellular heterogeneity, tumor cell migration, and response to therapies.

Genetically modified models

Benign tumors.—Tuberous sclerosis complex (TSC) is a genetic neurocutaneous disorder characterized by multiple benign hamartomas in various organ systems as well as severe neurological symptoms including seizures. Mouse models of TSC have offered substantial insight into a number of disease phenotypes, such as epilepsy and altered neuronal differentiation [127-129]; however, almost all lack the hallmark cortical tubers [130-132], and those with focal lesions lack histopathologic findings, such as true giant cells and astrogliosis [133, 134]. Cortical organoid models with mutations in TSC1 or TSC2 have dysregulated mTORC1 signaling during neurogenesis, forming cortical tuber-like structures with large numbers of astrocytes, which can be attenuated with mTORC1 inhibition [135].

Malignant tumors.—Tumorigenesis is thought to be initiated by a series of genetic events leading to the gain of cancer hallmarks, such as uncontrolled proliferation and ability to invade normal tissues. Genetically defined models of cancer offer the opportunity to interrogate the relationship between specific mutations and tumorigenesis, as well as a tumor model with a defined and controlled genetic background. Introduction of a mutant HRas G12V by homologous recombination into the gene body of TP53 in cerebral organoids leads to generation of rapidly proliferative cells that invade and take over the entire organoid [136]. These cells demonstrate a mesenchymal gene expression signature and are tumorigenic and invasive upon orthotopic xenograft into immunodeficient mice. Another model features MYC overexpression and $CDKN2A^{-/-}$/$CDKN2B^{-/-}$/$EGFR^{OE}$/$EGFRvIII^{OE}$ to yield rapidly proliferative cells that could expand and invade upon xenograft into immunodeficient animals [137]. These organoids responded to targeted therapies in a mutation-dependent manner, validating that these mutations were indeed driving tumor-like behavior and that these organoids could be used to study drug effects in the context of specific genomic variants.

Patient-derived models

Organoids generated from patient-derived cells and tissue may better recapitulate the genetic and cellular features of the originating parent tumor as compared with genetically engineered models. In one glioblastoma (GBM) organoid model, patient tissue is dissociated or minced and then embedded into Matrigel in the presence of EGF/bFGF to yield organoids that can be readily expanded and xenografted into immunodeficient mice [138]. These organoids responded to treatment with the MSI1 inhibitor, Luteolin, and Zika virus infection [139, 140]. In this same model, quiescent H2B-GFP cells were identified after doxycycline induction and long term chase periods and found to exhibit a mesenchymal-like gene expression pattern that was characterized by metabolic adaptions and extracellular matrix interactions as well as signatures of hypoxia and TGFP signaling [141]. A new GBM

organoid model was recently developed by cutting tumor tissue into small pieces that were cultured in a chemically defined medium without addition of EGF/bFGF or extracellular matrix [142, 143]. These organoids maintain cellular heterogeneity and exhibit many features similar to the original tumor, including histology, transcriptome, and genetic mutation landscapes. These organoids also show rapid and aggressive infiltration once transplanted into the brain of immunodeficient mice, and they have been used to test personalized treatment responses to targeted drug therapies and immunotherapy. Additional GBM organoid models have been developed by introducing GBM cells into 3-D printed neural organoids or iPSC-derived brain organoids [144-146]. By placing tumor cells within a neuroanatomically appropriate human microenvironment, these hybrid organoids may better recapitulate the intrinsic cellular states found in GBM [147]. The biology of GBM is heavily influenced by its microenvironment, and inclusion of these cellular components, either by preserving these cells from patient-derived tissue or by introducing them exogenously, will help to generate a more complete model of this disease.

Prospective

Brain organoids are a model system still in early phases of development, establishment, and application. Many methodology-oriented studies advocate for the inherent validity of brain organoids with a combination of imaging, transcriptomic, and electrophysiological analyses. This multifaceted approach is largely convincing; however, organoid data are rarely, if ever, a perfect match with existing datasets of the developing human brain. This is hardly surprising for numerous reasons, such as that organoids are generated *in vitro* without many essential components, including a blood supply and endothelial cell capillary network as well as functional immune system and microglia.

Complementing this first-principles approach, numerous studies have shown evidence of brain organoid empirical validity across a wide range of diseases. For example, brain organoids of congenital and infectious microcephaly show phenotypes of reduced organoid size and reduced progenitor proliferation, which are consistent with clinical observations and known disease processes [11]. As another example, patient-derived brain tumor organoids show *in vitro* drug sensitivity that is consistent with their genetic features and *in vivo* responses [142]. These kinds of empirical observations and clinical correlates contribute strongly to the evidence that these organoid models can recapitulate certain important aspects of the disorder with reasonable fidelity, and thus they lend confidence to the application of brain organoids in modeling neurological disorders.

Brain organoids offer some unique opportunities over other existing model systems, such as control over the genetic background, structural architecture, and intrinsic cell-type diversity. Stem cell technologies have allowed for access to human- and patient-specific samples, and ongoing studies, especially those of mental disorders without a known genetic etiology, would benefit from models derived from idiopathic cases. Organoid structure is well characterized by imaging methods as there are established immunohistochemical markers delineating key cytoarchitectural landmarks, and advancements in 3D volumetric imaging, such as those involving tissue clearing methods, may offer additional insights. The cellular diversity in brain organoids represents another unique advantage of this model system, and

advances in multi-modal and massively multiplexed single-cell technologies will allow for better characterization of the cell type-specific effects of different disease processes. Electrophysiology and network connectivity are less commonly examined in brain organoids, and future studies may benefit from specialized tools and instrumentation suitable for these relatively small 3D structures. Overall, advancements in analytical methods across a broad range of modalities will allow for better leveraging of the unique advantages and attributes of brain organoids. Correspondingly, the broadening repertoire of brain organoids across different developmental stages and specific brain regions will also help in the study of different neurological disorders within the best possible biological context.

In addition to these technical challenges that are currently being addressed by this rapidly growing field, brain organoids face some intrinsic limitations in modeling neurological disorders. Organoid models of neuropsychiatric disorders have so far been focused on the mechanistic connections between the underlying genetic alterations and the observed cellular and molecular changes. However, some of the major challenges are that these disorders can manifest in behavioral phenotypes that are likely impossible to assess in any *in vitro* system, and the broad clinical relevance of observed organoid phenotypes are unclear. Nonetheless, brain organoids already represent a major leap forward in modeling neurological diseases, and forthcoming innovations as well as growing application of this model system to a broad range of disease contexts will undoubtedly yield exciting new findings.

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Table 1.

Organoid models for congenital diseases with genetic etiologies

Disease class	Disease	Mutation	Patient derived/ genetically engineered	References
Structural phenotypes	Microcephaly	ASPM (MCPH1)	Patient-derived	[21]
		WRD62 (MCPH2)	Genetically engineered	[20]
		CDK5RAP2 (MCPH3)	Patient-derived	[10]
		CPAP (Seckel syndrome)	Patient-derived	[22]
		CHMP1A (Microcephaly with pontocerebellar hypoplasia)	Genetically engineered	[23]
		TREX1 (Aicardi-Goutières syndrome)	Patient-derived & Genetically engineered	[24]
	Lissencephaly	17p13.3 deletion (Miller-Dieker syndrome)	Patient-derived	[27, 28]
		LIS1	Genetically engineered	[31]
		KATNB1	Genetically engineered	[25]
		TUBA1A	Genetically engineered	[26]
	Periventricular heterotopia	PLEKHG6	Genetically engineered	[32]
		DCHS1 FAT4	Patient-derived & Genetically engineered	[33]
Neuropsychiatric disorders	Schizophrenia	DISC1	Genetically engineered	[40]
			Patient-derived	[18, 42]
		16p13.11 microduplication	Patient-derived	[44]
		FGFR1	Patient-derived	[45]
	Autism spectrum disorder	Idiopathic	Patient-derived	[47]
		CHD8	Genetically engineered	[48]
		CACNA1C (Timothy syndrome)	Patient-derived	[49]
	Intellectual disability	Trisomy 21 (Down syndrome)	Patient-derived	[57]
		MECP2 (Rett syndrome)	Patient-derived	[58]
Other	Gangliosidosis	GLB1 (GM1 gangliosidosis)	Genetically engineered	[59]
		HEXB (Sandhoff disease / GM2 gangliosidosis)	Patient-derived	[60]
	Pelizaeus-Merzbacher disease	PLP1	Patient-derived	[61]

Table 2.

Organoid models for congenital diseases with environmental exposures

Disease class	Exposure	References
Infection	Zika virus	[11, 67, 68, 70-83, 148]
	Japanese encephalitis virus	[84]
	Cytomegalovirus	[86, 87]
Chemical agents & radiation	Hypoxia	[89-91]
	Alcohol	[92]
	Cocaine	[93]
	Nicotine	[94]
	5-MeO-DMT	[95]
	Tranylcypromine	[102]
	Imadazole antifungals	[103]
	Bisphenol A	[11]
	Gamma irradiation	[104]

Table 3.

Organoid models for neurodegenerative diseases

Disease	Mutation	Patient derived/ genetically engineered	References
Alzheimer's disease	APP duplication	Patient derived	
		Patient derived	[114]
	PSN1	Patient derived	[116]
		Patient derived	
	Trisomy 21	Patient derived	[115]
	APOE4	Genetically engineered	[117]
		Genetically engineered	[118]
	Wild type with Aftin-5 treatment	Patient derived	[119]
Frontotemporal dementia	MAPT	Patient derived	[121]
Huntington's disease	HTT	Patient derived	[124]
Creutzfeldt-Jakob disease	Wild type with CJD brain homogenate exposure	Patient derived	[125]
Hereditary spastic paraplegia	SPG11	Patient-derived	[126]

Table 4.

Organoid models for cancer

Disease	Mutation	Patient derived/ genetically engineered	References
Tuberous Sclerosis	TSC1 & TSC2	Genetically engineered	[135]
Glioma	HRAS & TP53	Genetically engineered	[136]
	MYC, CDKN2A, CDKN2B, EGFR, EGFRvIII	Genetically engineered	[137]
	Various	Patient derived	[138-142, 144-146]