REVIEW

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Brain Organoids as Tools for Modeling Human Neurodevelopmental Disorders

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Brain organoids recapitulate in vitro the specific stages of in vivo human brain development, thus offering an innovative tool by which to model human neurodevelopmental disease. We review here how brain organoids have been used to study neurodevelopmental disease and consider their potential for both technological advancement and therapeutic development.

stem cells; organoids; neurodevelopmental disease; disease modeling

Introduction

Investigating neurodevelopmental disease pathogenesis presents considerable challenge due to limited accessibility of human central nervous system (CNS) tissue and poor recapitulation of animal models. The considerable variability in neuroanatomy and connectivity that exists between individuals because of disparate genetic background and environmental exposure (40) introduces another barrier to understanding disease progression. Our knowledge of cellular phenotypes in neuropathological conditions has historically derived primarily from postmortem analysis of CNS tissue, but tissue represents only a single disease time point and may not be well preserved. Functional neuroimaging and animal models have provided noninvasive alternatives for modeling human neurological diseases, but these models are limited by age, sex, pathological heterogeneity, and inconsistent translatability between species (84) (FIGURE 1).

Recent advancement of stem cell technology has introduced a new model by which to study the human brain. Human embryonic stem cells (hESCs) can be induced into neural stem cells and further differentiated into neurons and glia. Due to their ethically controversial origin, however, hESCs have been only loosely adopted. Cellular reprogramming—a technique that reverts patient-specific somatic cells to a pluripotent state, the induced pluripotent stem cells (iPSCs) (122)—offers a way around this problem. iPSCs carry the genotype of the patient donor and, like hESCs, can be differentiated into many different cell types, including neurons and glia. iPSCs thus enable direct in vitro manipulation of relevant cellular phenotypes affected in nervous system diseases.

Initial efforts to model neurological diseases in vitro consisted of neuronal culturing in monolayer. Neurons and glia can be obtained by differentiating neural progenitor cells (NPCs) that are, in turn, directed from iPSCs or ESCs by dual SMAD inhibition (20). NPCs self-organize into rosettes resembling the embryonic neural tube—thereby mimicking in vivo neocortical development—and are thus a predominant method in several differentiation protocols (93). Alternatively, neurons can be induced directly from fibroblasts via forcible expression of several transcription factors—Brn2, Ascl1, and MytL1—or even a single transcription factor, Ngn2 (17, 90, 140). Regardless, culturing neural cells in a 2D environment limits the opportunity for cells to create the structure and organized network connectivity observed in vivo (56). The need for improved in vitro models that more accurately recapitulate human brain complexity and overcome the limitations of 2D models led to the development of 3D brain organoids (FIGURE 1).

The founding discovery of what would come to be called an "organoid" was made by the Sasai laboratory, a pioneering stem cell research group upon whose shoulders all subsequent organoid research stands. They observed that ESCs and iPSCs could self-organize and aggregate in a manner remarkably recapitulative of in vivo development (32, 85). Indeed, although the Sasai group's initial monographs detailed the self-formation of optic tissue (32, 85), organoids of other neural fate likewise display highly similar organization, gene expression profiling, topographical induction, and temporal development to that seen during fetal neural development (18, 19, 61, 68, 134). Currently, brain organoids can be generated in two ways: non-patterned or patterned. Non-patterned organoids, generally grown embedded in an extracellular matrix, self-organize into different brain regions via endogenous patterning cues (61, 101). Patterned organoids, in contrast, are differentiated into specific brain regions—forebrain or midbrain, for example—by adding external growth factors (74, 92, 98).

Brain organoids, patterned or otherwise, confer investigative ability unavailable in vivo. Evaluation of normal cellular migration and organoid maturation

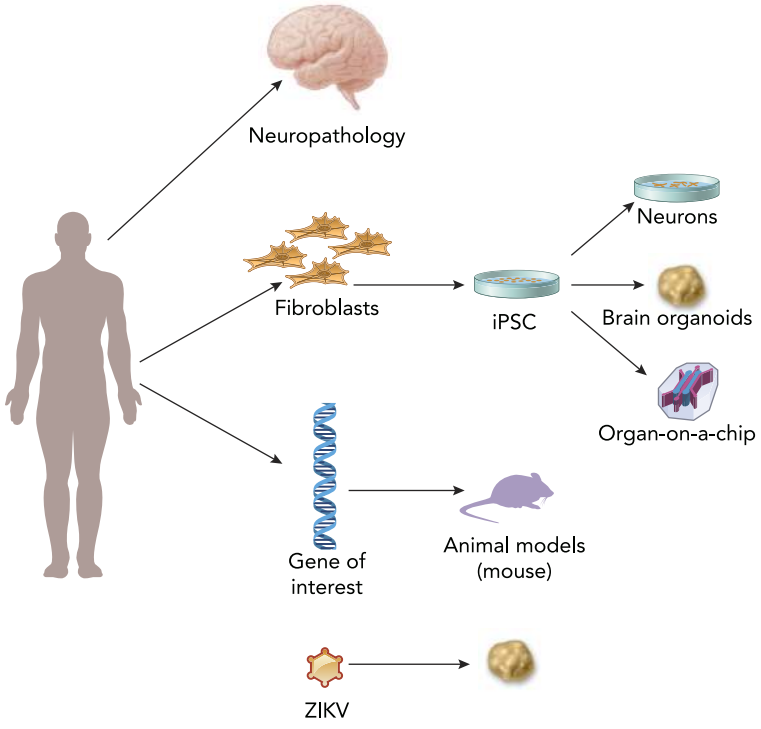
with immunofluorescence and live imaging can be used to examine tissue stratification and to detect early signs of pathology (11, 18). Genomic engineering tools can modify patient-derived stem cells to investigate gene-function relation (114), regionally patterned organoids can be fused to investigate complex interregional dynamics (11, 114, 135), and patch-clamp electrophysiology and optogenetics can assess functional integration (70) (FIGURE 2). Furthermore, recent detection of spontaneous network activity with regular oscillatory waves, similar to that observed in preterm human electroencephalography, demonstrated the capacity of multi-electrode array to dynamically assess network activity in long-term mature organoids (127). The accessibility of iPSCs and brain organoids has thus introduced unprecedented possibility for in vitro neurodevelopmental disease modeling.

Applications of Brain Organoids as Neurodevelopmental Disease Models

Perhaps the greatest application of brain organoid technology thus far, in vitro modeling of neurodevelopmental disease enables observation of disease progression throughout neurodevelopment and—in conjunction with novel genetic techniques—the opportunity to interrogate underlying pathological mechanisms with previously precluded precision. The versatility of brain organoids permits modeling diseases of either intrinsic (i.e., genetic) or extrinsic (i.e., environmentally mediated) etiology (FIGURE 2). However, despite recent characterization of functional network development (127), developmental disorders in which gross structural abnormalities predominate remain the more accessible for in vitro modeling.

Autosomal Recessive Primary Microcephaly

Autosomal recessive primary microcephaly (MCPH)—a genetic form of microcephaly, itself a clinical entity of heterogeneous etiology—has been linked to genetic mutations in neurodevelopmental pathways (82). Individuals with MCPH clinically portray nonprogressive intellectual disability and neuropathologically exhibit microcephalic brains, with reduction concentrated in the cerebral cortex (132). MCPH has been modeled with organoids generated from patient-derived iPSCs carrying mutation(s) in either *ASPM*, the gene that codes for a protein involved with mitotic spindle function and that accounts for a plurality of MCPH cases (36), or *CDK5RAP2*, a gene whose product localizes to the mitotic spindle pole during neurogenesis (61, 63, 132). Those iPSCs in which *ASPM* expression was downregulated, predicted to impede neural progenitor proliferation, yielded hypoplastic organoids with fewer proliferative cells, decreased



Advantages	Disadvantages
• Definite pathophysiology	• Single snapshot • Limited sample availability
• Reproducible	• Structurally simple
• Architecturally complex • Cellular heterogeneity	• Poor long-term postnatal recapitulation
• Precise experimental control	• Physiologically constrained
• Complex disease phenotype	• Inadequate translatability
• Accessibility • Causal manipulation	• Reductionist mechanistic analysis

FIGURE 1. **Advantages and disadvantages of methods of neurodevelopmental disease analysis**
Neurodevelopmental disease mechanisms can be assessed using various modalities, each of which has unique and complementary strengths, with selection dependent on desired readout.

neocortex-like morphology, and diminished neuroepithelial structural integrity (63). Functional analysis revealed calcium activity in fewer cells than the controls—implicating neuronal maturation impediment—and decreased synchrony (63). *CDK5RAP2*-mutant organoids likewise portrayed hypoplasticity with sparse progenitor and neuroepithelial regions (61). Coincident findings of premature neural differentiation and increased neuron quantity were supported by observation of increased neuronal differentiation upon *CDK5RAP2* RNAi-knockdown (61). Successful phenotypic rescue upon electroporated expression of CDK5RAP2 protein confirmed viable in vitro recapitulation of MCPH (61).

Microcephaly in Seckel Syndrome

Seckel syndrome—a disorder argued to be on a clinical spectrum with primary microcephaly and whose clinical features include severe pre- and postnatal growth restriction, microcephaly, and intellectual disability (33, 71)—is observed in individuals with mutated centrosomal-P4.1-associated protein (CPAP). Concordant with other microcephalies, organoids of Seckel syndrome patient-derivation featured diminished size with reduced neuroepithelium and disordered progenitor regions (38). In argument for organoids' biologically intrinsic mechanistic value, Gabriel et al. (38) identified a ciliary role in NPC sustenance and a regulatory role of ciliary length by CPAP. Indeed, detailed interrogation of Seckel organoids revealed more numerous and longer apical progenitor cilia compared with controls (38). Their work thus demonstrated that, in addition to modeling structural deficits, brain organoids enable investigation—in vitro—of molecular mechanisms underlying neurodevelopmental disease.

Macrocephaly

Just as some mutations confer a microcephalic phenotype, others—*PTEN* loss-of-function, for example—have macrocephalic consequence (15). Cortical organoids generated from *PTEN*-knockout hESCs exhibited increased neuroepithelial outgrowth, surface area, and volume (64). Most striking, however, was the enhanced gyrification evident in *PTEN*-mutant organoids. At the cellular level, *PTEN*-mutants displayed more proliferative cells throughout development, an increase reflected in expanded neural progenitor pools in the ventricular and subventricular zones. It was over these pools of increased proliferative cellularity that the neuroepithelial curvature was more pronounced, an observation later supported biophysically (55). Viral reintroduction of *PTEN* offered successful phenotypic rescue, with normal neural differentiation and proliferation and the generation of smooth organoids. Indeed, manipulating AKT signaling effected a phenotypic dose response: less AKT signaling yielded smaller, smoother cortical organoids, and increased AKT signaling generated larger organoids with increased fold density (64).

Congenital Lissencephaly

Lissencephaly is a clinical entity characterized by decreased or absent gyration resulting from deficient neuronal migration during development (49). Heterozygous chromosome 17p13.3 deletion results in Miller-Dieker syndrome (MDS), a neurodevelopmental lissencephalic disorder whose clinical characteristics include intellectual disability and seizures (13). This deletion often involves *LIS1* and *YWHAE*, the gene products of which are components of a multiprotein complex that has regulatory function of cytoskeletal protein dynamics (133). Because the LIS1 multiprotein complex is involved with the mitotic spindle and radial glia proliferation, protein defects within the complex yield structural defects during development (137). Organoids developed from MDS patient-derived iPSCs exhibited smaller size and slower expansion compared with controls, and likewise featured

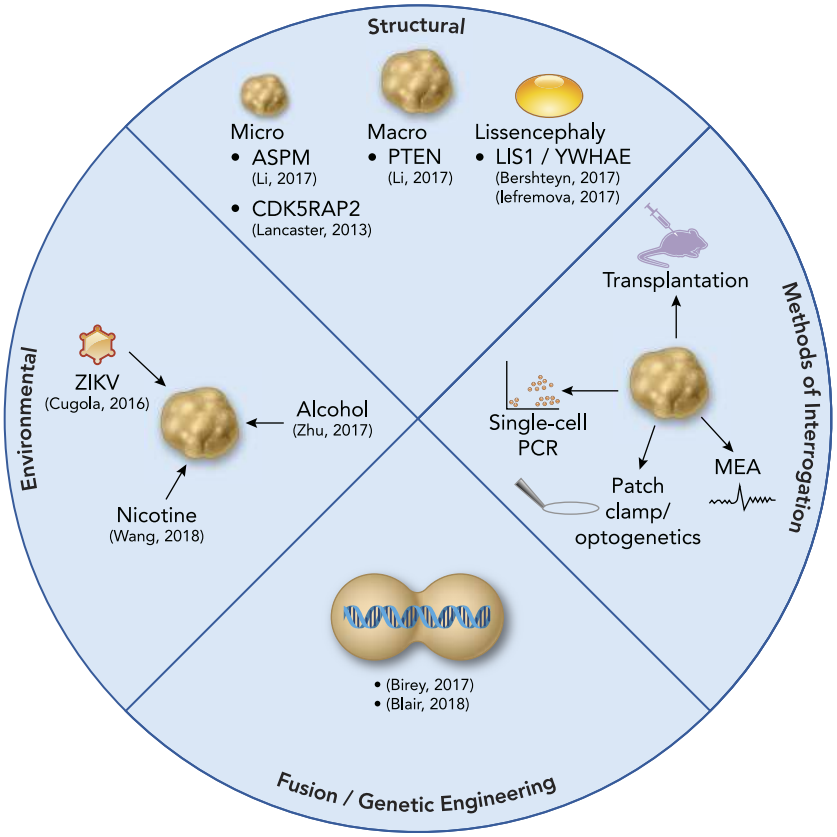


FIGURE 2. **Brain organoid disease modeling and analytic techniques**
Brain organoids can model congenital structural deficits or be subjected to environmental insult. In addition, genetic engineering and multi-organoid fusion enable the assessment of a broader array of disease mechanisms, such as abnormal interregional development. Various methods can be used to evaluate the developmental changes that underlie the disparate phenotypes observed between normal and diseased organoids.

structural aberrancies in neuroepithelial loops, including reduced apical and basal membrane length, loop diameter, and overall size of a ventricle-like space (50). Interrogation of MDS organoids at finer resolution revealed disrupted radial glia cytoskeletal networks with diminished projection of truncated microtubules toward the basal membrane (50). Moreover, mitotic spindle cleavage in MDS organoids exhibited a non-random planar switch (from vertical to horizontal and asymmetric) and disrupted adhesion molecules in the ventricular zone. Reduced β-catenin expression in organoids and influential N-cadherin signaling in MDS patient rosettes implicated Wnt pathway disruption in MDS; pharmacological β-catenin activation via CHIR99021 inhibition of GSK3β rescued division plane orientation, cortical loop structure, and apical membrane length (50). Notably, expressing LIS1 or 14-3-3ε (the protein product of *YWHAE*) in MDS patient cells offered partial restoration of a control phenotype (50).

Individuals with MDS exhibit pronouncedly disrupted cortical cellular distribution: immature neurons appear in the deepest cortical layer, and cell proliferation markers imply disruption of neural progenitor proliferation (112). Organoids from MDS patients—developed to investigate neural progenitor defects in vitro—revealed a disrupted ventricular zone surface, disproportionately frequent horizontal cleavage angles, and outer radial glia with longer mitotic delay before cytokinesis (9). Despite intact migratory processes, MDS organoids exhibited impaired neural migration such that migration was initiated but not sustained and was observed to be slower and less linear. Remarkably, restoration of the LIS1 and 14-3-3ε proteins completely rescued phenotypic aberrancy to wild-type (9).

Karzbrun et al. (55) sought to define the physical forces that regulate cortical folding and then apply that understanding to a lissencephalic organoid model. Physically, organoid surface wrinkling appeared to be subject to the mechanical instability instigated by compression, such that wrinkling starkly emerged when the nuclear density reached a threshold (55). Application to a heterozygous *LIS1* mutant organoid model revealed a wider range of cortical thicknesses than control organoids and the longer wavelengths (i.e., distance from one wrinkle crest to another) and decreased curvature expected of lissencephaly (55). *LIS1* mutants exhibited decreased expression of cytoskeletal genes, and assessment of ESCs and NPCs with atomic force microscopy indicated the *LIS1* mutant cells were twice as elastic as their control counterparts (55). Increased elasticity would be expected to confer resistance against a compressive force inducing folding and might partially explain the decreased gyrification of lissencephaly. Such a model would likewise be consistent with a separate group's observation of increased neuroepithelial curvature in areas with more proliferating cells (64).

Sandhoff Disease

Sandhoff disease is an autosomal recessive lysosomal storage disorder in which mutant-*HEXB* insufficiency of β-hexosaminidase causes lysosomal accumulation of GM2 ganglioside (104, 105). The GM2 gangliosidoses clinically feature developmental delay or regression, seizures, macrocephaly, hypotonia, and progressive decline in motor and cognitive functioning (3, 124). Allende et al. (3) developed organoids from a Sandhoff disease patient and from CRISPR/Cas9-corrected *HEXB* mutant isogenic iPSCs. Compared with *HEXB*-corrected organoids, Sandhoff disease patient organoids appeared macrocephalic, with increased proliferation and dysfunctional differentiation (3). By *week 4* of growth, immunostaining revealed GM2 ganglioside accumulation and even inclusion body development in Sandhoff disease organoids; *HEXB*-corrected organoids accumulated significantly less GM2 ganglioside (3). In evidence of phenotypic rescue, restoration of β-hexosaminidase via AAV-*HEXA/B* injection decreased Sandhoff disease organoid size and lessened GM2 accumulation (3).

Tuberous Sclerosis Complex

Tuberous sclerosis complex (TSC) is a multisystem developmental disorder in which heterozygous germline mutations in *TSC1* or *TSC2*—encoding hamartin and tuberin, respectively—cause constitutive activation of mechanistic target of rapamycin complex 1 (mTORC1) (95). The wide-ranging clinical features of TSC include seizures, autism, and intellectual disability; facial angiofibromas; cardiac rhabomyomas; and, most characteristically, cortical tubers (28). Blair et al. (12) sought to investigate the underlying genetic mechanisms of cortical tuber development—the proposed of which include "second hit" loss of heterozygosity, abnormal retrotransposition, and haploinsufficiency (52, 75)—using cortical spheroids generated from CRISPR/Cas9-mutated (both hetero and homozygously) *TSC1* or *TSC2* hESCs. Homozygous *TSC2*-knockout spheroids exhibited persistently upregulated mTORC1 during neuronal differentiation, a period customarily of quiescent mTORC1 signaling (12). Homozygous *TSC1* or *TSC2* knockouts additionally featured enhanced mTOR-dependent, gliogenic JAK-STAT signaling: treatment with the mTOR inhibitor rapamycin reduced pathway activation, decreased astrocytic GFAP expression, and increased expression of neuronal markers (12). Blair et al. (12) then engineered

hESCs with a Cre-inducible *TSC2* mutation to assess the verity of the second-hit hypothesis. Homozygous, but not heterozygous, spheroids exhibited enlarged, misshapen neurons, increased glial cells, and hypertrophic, filamentous "giant cells" resembling those characteristic of cortical tubers. Spheroids developed from a heterozygous TSC patient, reprogrammed from fibroblasts and engineered to receive a second hit, subsequently confirmed the necessity of biallelic inactivation to develop the dysmorphic cells characteristic of TSC. Just as rapamycin earlier reduced JAK-STAT signaling to combat mTORC1 overactivation, so, too, could it reduce cellular enlargement and recover neuronal—rather than glial—differentiation. A developmental time dependency constrained treatment efficacy, however, such that rapamycin administered too late offered insufficient phenotypic rescue (12).

Rett Syndrome

Rett syndrome is a neurodevelopmental disorder almost universally caused by X-linked mutations in *MECP2*, the gene encoding methyl-CpG binding protein 2. Clinically, females with Rett syndrome typically experience normally appearing development for 12–18 mo followed by regression—in domains such as hand movement and language—and the onset of gait abnormalities (51, 86); males experience severe congenital encephalopathy and early death (108). Rett patient-derived cells exhibit structural and connectivity defects (73), and a suppressive role of MeCP2 has been implicated in posttranscriptional micro-RNA processing essential for neural development (22). Brain organoids generated from Rett syndrome patients revealed upregulated miR-199 and miR-214, two microRNAs that are involved in ERK and AKT signaling pathways for neurogenesis and neural differentiation (78). Moreover, Rett mutant organoids displayed increased ventricular area with decreased ventricular wall thickness, consistent with increased neural progenitors and proliferation with concomitantly impaired neurogenesis (78).

Autism Spectrum Disorder

The term autism spectrum disorder (ASD) describes a set of clinically heterogeneous disorders whose core characteristics include behavioral stereotypies and impairments in social interaction and language (1). ASD is thought to be of both genetic and environmental etiological derivation (100), and, despite the wide-ranging concordance estimates observed for twins, the estimated heritability of ASD is 0.7–0.8 (26, 118). Perhaps underlying the broad clinical heterogeneity, ASD is associated with a broad array of genetic profiles and aberrancies—from widely varying allelic frequencies (e.g., common, rare, etc.) to disparate inheritance patterns (e.g., dominant, X-linked, etc.) to variant type (e.g., insertion/deletion, copy number variant, etc.) (100). Despite this genetic heterogeneity, autism may represent a convergence phenotype of several common pathways (39); in vitro cellular modeling may offer a versatile modality by which to interrogate these various underlying mechanisms (43, 67, 72).

ASD may be attributable to heterogeneous intercellular transcriptomic changes with convergent cellular and molecular pathways (99), one of which may be excitatory/inhibitory imbalance (103, 123). Cortical organoids using iPSCs drawn from macrocephalic ASD individuals revealed, compared with unaffected family members, transcriptomic differences in cell fate and proliferation; cytoskeletal regulation of dynamic cytologic growth, guidance, and maintenance; synaptic assembly and channel functioning, such as potassium ion channel upregulation and ligand-receptor interactions; and upregulated GABAergic enzyme synthesis (74). Despite the low number of individuals tested, the similar findings observed in an independent cohort of ASD individuals increase confidence in the data (72). Cytologic analysis exhibited increased VGAT+ inhibitory synapse formation with concomitantly increased GABAergic neural fate, a finding subsequently confirmed electrophysiologically (74). Transcriptomic upregulation correlated with autism symptom severity, and one of the principal genes upregulated throughout development was *FOXG1*, the product of which is a developmental transcription factor for the telencephalon and mutations in which confer neurodevelopmental dysfunction (4, 15, 53, 74, 79, 97). Remarkably, interference with *FOXG1* expression could revert the GABAergic neural production in ASD organoids to normal (74). Evaluation of another gene frequently involved in autism, the chromatin-remodeling factor *CHD8* (chromodomain helicase DNA-binding protein 8), likewise revealed upregulation of transcripts used for GABAergic interneuronal development (130). Although ASD is associated with an excitatory/inhibitory imbalance, the concordant findings of GABAergic increase in these models contrasts with evidence indicating GABAergic reduction in ASD (21, 34).

Microglia in Organoids

Concomitantly with imbalanced excitation and inhibition, individuals with ASD and other neuropsychiatric disorders are proposed to have aberrant synaptic structure and connectivity (7, 48, 73, 94, 126). Microglia function in synaptic remodeling during development (77, 107, 117, 120) and may be involved in ASD and other neurodevelopmental

disorders (35, 59, 91, 106). For example, ASD patients exhibit increased microglia density and microglial morphology indicative of activation (81, 121, 125), and wild-type microglial engraftment could impede disease progression and improve phenotype in a Rett syndrome mouse model (31). Indeed, rodents have, to date, been the primary model for studying microglia (10, 117); however, murine and human microglia are thought to differ markedly (111a). Recent derivation of microglia from hiPSCs offers a novel opportunity by which to study microglial function in neurological disease (2, 83). Two groups separately derived microglia-like cells from human iPS cells that could, in organoid co-culture, integrate and respond to either laser- or needle-induced traumatic injury (2, 83), and a third group induced an inflammatory response by exposing neurospheres co-cultured with human microglia to bacterial lipopolysaccharide and flaviviral infection (80). Whereas it had been thought that microglia—of primitive myeloid progenitor yolk-sac derivation (42)—must be introduced to neuroectodermally derived organoids, recent modification of an undirected-differentiation brain organoid protocol, in which all three primitive germ layers are present in early organoidogenesis, revealed intrinsic development of Iba-1-positive microglia from mesodermal precursors (89). Such inclusion of microglia in organoids promises expanded opportunities for disease modeling.

Teratogens Modeling

In addition to genetically based neurodevelopmental disease modeling, the in vitro accessibility and manipulability of brain organoids has also facilitated their use in studying CNS teratogens. Alcohol consumption during pregnancy, perhaps the foremost CNS teratogen, is a major public health concern (111). Clinically, fetal alcohol spectrum disorder features developmental delay, behavioral impairment, and cognitive dysfunction (102). The neuropathological consequences of prenatal alcohol exposure include decreased brain volume and thinning of the corpus callosum (reviewed in Ref. 62). Exposure of brain organoids to alcohol impaired neural maturation, reduced neurite outgrowth, and increased cell death (141). Nicotine is another common teratogen, fetal exposure to which can disrupt development and increase long-lasting health risks for the offspring even into adulthood (14). Brain organoids exposed to nicotine showed impaired neuronal migration and differentiation (131).

As with toxin exposure, viral insult also presents an external mechanism by which neural development can be impaired (6). Exposure to Zika virus in utero has been associated with an increased frequency of congenital microcephaly (88). A causal mechanism underlying this association was established by infecting brain organoids and a mouse model with a Brazilian strain of Zika virus (29). Consistent with the microcephaly observed in mice, the virus depleted the neural progenitor cell pool of organoids by preferentially infecting NPCs, causing cell death, and disrupting the cortical plate (29).

Fusion, Genome Engineering, and the Next Generation of Organoid Technology

Modeling neurological disorders in the past has often required animal or other model systems, and neurodevelopmental disorders present a particular challenge due to limited accessibility. The enhanced breadth and depth of organoid technology using human cells, however, has facilitated the modeling of an ever-increasing array of disorders with both unprecedented recapitulative accuracy and genetic manipulative capacity. Multi-organoid fusion, genomic engineering, and methods of vascularization promise further advancement in disease modeling and pharmacological development.

Multi-Organoid Fusion

Development of the nervous system is a complex yet synchronous dance of multi-modal events, including neuronal migration and the establishment of synaptic interconnectivity. Although organoid modeling methods to date recapitulate neural development remarkably well (61, 98), the disrupted neuronal migration and regional interconnectivity underlying some neurological disorders argues for methods that can model these actions. Fusing together organoids of different pre-specified brain regions offers a modality by which to model such interregional interaction (5, 11, 134, 135). Birey et al. (11) pre-patterned and fused pallium and subpallium spheroids using patient-derived cells to identify GABAergic interneuronal migration deficits present in Timothy syndrome. Timothy syndrome—a multisystem neurodevelopmental disorder resulting from defective L-type calcium channels (LTCC) secondary to mutations in their encoding gene, *CACNA1C* ($Ca_V1.2$)—clinically includes features such as cognitive impairment and autism, cardiac defects, syndactyly, and immune deficiency (119). Assessment of interneuronal migration in fused spheroids revealed increased neuronal saltation frequency but decreased length and speed (11). Because Timothy syndrome results from gain-of-function mutation, adding the LTCC blocker nimodipine to patient-derived cells rescued the defective saltatory phenotype (11).

Multi-organoid fusion thus offers a viable means by which to model interregional developmental dynamics as well as phenotypic defects associated with disease.

Genome Engineering

Novel techniques in genomic engineering—including zinc-finger nucleases, transcription activator-like effector nucleases (TALEN), and the CRISPR/Cas9 system (23, 45, 46, 54, 69, 113a)—have enhanced our ability to manipulate the human cellular genome with unprecedented precision (16, 58). The considerable genetic heterogeneity underlying neurological disease demands techniques by which to more efficiently define the effects of genetic variants on neurodevelopmental pathways and phenotypic change (76). These new techniques have enhanced the utility of in vitro disease modeling by enabling pathogenic mutations to be introduced into wild-type stem cells or mutations to be corrected in patient-derived stem cells (113, 128). Despite concern of off-target effects and inefficiency (37, 65, 69, 139), whole-genome sequencing suggests these effects may be less prevalent in iPSCs than feared (116, 129), and updated genome editing strategies—such as target selection and guide protein modification—have sought to minimize these effects (24, 25, 47, 57, 69, 113a, 130, 139). In vitro organoid modeling of several of the diseases featured in this review has only been successful because of genomic manipulability, and further genomic innovation promises disease modeling with even greater complexity—sporadic diseases, for example (44)—and portends enhanced clinical translatability (109).

Transplantation, Vascularization, and Pharmaceutical Development

One of the primary factors limiting further development of organoid technology has been size restriction imposed by insufficient nutrient delivery to the organoid interior due to the absence of vascularization (41, 136). One solution is to appropriate in vivo nutrient distribution machinery (30, 70). Brain organoids engrafted into the retrosplenial cortex of immunodeficient mice successfully vascularized, yielding coincidently increasing graft surface area with minimal or absent apoptosis (70). The engrafted organoids retained differentiability, underwent astrocytic and oligodendrocytic gliogenesis, and functionally integrated (70). Another solution—separate differentiation of patient-derived iPSCs into cortical organoids and endothelial cells with subsequent co-culture—likewise exhibited organoid vascularization (96).

Much of the immediate clinical utility expected of the cortical organoid system is its pharmaceutical potential; the true efficacy of such a model, however, demands vasculature and a blood-brain barrier (BBB). To that end, a vascularized 3D model has been used for multi-disciplinary interrogation of neurotoxicity (110), and dynamic BBB spheroids have been employed to model drug transport and neurotoxicity (24, 87). Following co-culture of endothelial cells, astrocytes, and pericytes, the spheroid exhibited an interior with preponderant astrocytes and an exterior surface of pericytes and endothelial cells (8). Critically, this external surface appeared dynamic, with regulated permeability, expression and activity of a P-glycoprotein efflux pump, and peptide receptor-mediated transcytosis (24). Successful development of these early BBB models suggests organoid technology—irrespective of BBB inclusion—may relieve dependence on imperfect animal models for pharmaceutical development and toxin interrogation, and may well confer future opportunity for personalized therapeutics (66, 110).

Future Challenges

Brain organoid technology has greatly enhanced neurodevelopmental disease research, but, despite its potential, many challenges and technical limitations remain. Organoid protocols may exhibit significant batch variability and can give rise to different compositions of brain regions (60); however, improvements aiming to reduce organoid heterogeneity and improve reproducibility are being developed (98, 114, 128, 138). Organoids are also limited by their minimal or lack of relevant cellular subtypes (e.g., microglia and endothelial cells), which restricts their resemblance of in vivo development to only early gestation (92, 98). Later neocortical development requires vascularization for nutrient diffusion, the absence of which results in interior necrosis (60). As was previously mentioned, however, novel vascularization techniques are initial steps to resolving this challenge (70, 96). Moreover, because most differentiation protocols favor ectodermal fate, microglia, of mesodermal origination, are usually absent. Improved methods of co-culturing or introducing these cells into brain organoids is necessary for accurate disease modeling. Last, to advance the translation of pathophysiological findings from organoids to the human brain, it is imperative to understand whether brain organoids create the neural circuitry observed in the human brain and address any ensuing ethical concerns. ■

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