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Stem Cell Based Organoid Models of Neurodevelopmental Disorders

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Abstract

The last decade has seen an explosion in the identification of genetic causes of neurodevelopmental disorders (NDDs), including Mendelian, de novo, and somatic, factors. These discoveries provide opportunities to understand cellular and molecular mechanisms as well as potential gene-gene and gene-environment interactions, to support novel therapies. Stem cell-based models, particularly human brain organoids, can capture disease-associated alleles in the context of the human genome, engineered to mirror disease-relevant aspects of cellular complexity and developmental timing. These models have brought key insights into NDDs as diverse as microcephaly, autism, and focal epilepsy. But intrinsic organoid-to-organoid variability, low levels of certain brain-resident cell types, and long culture times required to reach maturity can impede progress. Several recent advances incorporate specific morphogen gradients, mixtures of diverse brain cell types, and organoid engraftment into animal models. Together with non-human primate organoid comparisons, mechanisms of human NDDs are emerging.

Keywords

Brain Organoid; Assembloid; Neural Rosette; Mutation; Microcephaly; Autism; Epilepsy; Recessive; Dominant; Mosaic; Gene-Environment-Interaction; Genotype-Phenotype

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Financial Disclosures

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Online resources

Fuji/Cellular Dynamics to assess CIRM-funded patient-derived IPSC lines from patients with neurodevelopmental disease: https://www.fujifilmcdi.com/search-cirm/

Simons Foundation for Autism Research Initiative (SFARI)-funded patient-derived IPSC lines from patients with autism: https://www.sfari.org/resource/ips-cells/

NIMH repository and Genomics Resource (NRGR) patient-derived IPSC lines from patients with psychiatric conditions, including some NDDs https://explorer.nimhgenetics.org/subject-counts/explore/?c=ipsc

Matchmaker Exchange: https://www.matchmakerexchange.org

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Introduction

Neurodevelopmental disorders (NDDs) most often reflect defects in basic mechanisms of brain development including tissue patterning, neurogenesis, migration, cell survival and connectivity. NDDs are associated with abnormal brain function, most presenting clinically with features of autism, epilepsy, cerebral palsy and intellectual disability that can last a lifetime (1). Although some definitions of NDDs include less severe diagnoses like attention deficit hyperactivity disorder, dyslexia and tics that can associate with greater chance of normal long term neurological outcomes, this review is focused on severe forms of disease, more often associated with Mendelian mutations, and more likely to produce relevant brain organoid disease models.

NDDs incur an enormous toll on patients and families, accounting for up to 25% of all chronic pediatric diseases, and upwards of $500 billion annual total US healthcare costs and lost wages (2). Most NDDs show pleiotropic or complex syndromic features, often involving several organs in addition to the CNS (3). While a subset is due to chromosomal copy number or structural changes, most link to single-gene disorders or catastrophic environmental events such as hypoxic-ischemic encephalopathy (4), where there is a growing experience in organoid modeling.

Clinical evaluation of patients with NDDs historically include brain imaging and screening for a handful of metabolic and genetic conditions, but the past 10 years has seen widespread adoption of exome or genome sequencing. This has both dramatically increased diagnostic accuracy, uncovered thousands of new causes of disease, and led to a plethora of new genotype-phenotype correlations (5).

Focusing on the class of NDD that is severely debilitating, molecular diagnosis can now be made in 30~40% of patients across the NDD spectrum (6, 7), and consequently, thousands of unique single genes, chromosomal structural variants and somatic mutations linked to specific NDDs phenotypes (8). The NDD field has greatly benefited from a collaborative network of physicians and scientists sharing data to refine genotype-phenotype correlations through networks like Matchmaker Exchange, where rare mutations aggregate across the globe, and connect to family support groups. This genotype-driven rather than phenotype-driven path means that even patients in different parts of the world can easily connect. This decade-long experience provides the foundation for future disease modeling and hypothesis testing to understand pathological mechanisms and build preclinic models.

Given lifelong disabilities that accompany many NDDs, there is an enormous unmet medical need and opportunity to develop therapies. Historically therapies have been assessed in animal models, ranging from worm to mouse, and while these have been powerful, there is a growing appreciation for complementary use of human stem cell models to fill knowledge gaps. Moreover, use of patient-derived cells can avoid bottlenecks of genomic engineering often required to produce animal models. On the other hand, reprogramming patient cells, creating isogenic controls, and allelic series of mutations can itself be a bottleneck.

A range of new technologies applied to human brain organoid (hBO) NDD models can interrogate the genome, transcriptome, epigenome, proteome, and metabolome to uncover

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disease mechanisms. Coupled with *in vivo* models and a rich history of neuropathological assessment for many NDDs, there is growing clarity around specific phenotypes that recapitulate in various models. For instance, decades of neuropathology experience from patients with resected brain tissue during epilepsy surgery can help inform organoid models of disease (9). Further, disrupted gene regulatory mechanisms observed in stem cell autism patient-derived brain organoids have been at least partially validated in autism postmortem brain tissue (10). While access to limited postmortem brain tissues will likely remain a bottleneck, biobanks containing a range of NDD patient stem cell lines are likely to grow, and lead to greater opportunities to model disease.

Human brain organoids to study NDDs

hBOs derived from pluripotent stem cells are three-dimensional cellular aggregates that incorporate aspects of cellular diversity and structural architecture of the developing human brain. The first hBOs report by Sasai and colleagues describe both the methods of generation and diversity of cell types (11). Lancaster et al. further demonstrated that hBOs derived from iPSCs from patients with microcephaly often showed greatly diminished hBO size, one of the first demonstrations that hBOs could capture NDD phenotypes (12). Thus, the race to build ever more sophisticated and physiologically relevant models was on.

Mammalian stem cells differentiated towards neural lineages usually favor forebrain neural lineages, with an intrinsically programmed ‘clock’ to generate first deep layer and then superficial cortical layer neurons (13). These stem cells, cultured in chemically defined media, spontaneously generate polarized pseudostratified neural epithelium, forming neural rosettes (11). These neural rosettes are abundantly populated with radial glia-like cells surrounding the lumen that display characteristic inter-kinetic nuclear migration, thereby restricting mitotic divisions to the apical surface (14). The newly generated neural progenitors populate a subventricular-like zone containing some outer radial glia (oRG), a cell type that has greatly expanded evolutionarily in primate species (15). These aspects are particularly useful in modeling NDDs.

While self-organized hBOs contain a range of cell types including excitatory, inhibitory, and midbrain cells, more recent efforts have focused on both increasing hBOs cellular uniformity through the generation of patterned hBOs that mimics specific brain regions (16–18), or fusing two or more hBOs or cell types to create brain ‘assembloids (19). Forebrain organoids can be made more uniform through the application of specific chemicals or proteins that block pathways promoting other cell types, for instance, blockade of both TGF-β and BMP can prevent generation of mesendodermal and epidermal lineages (20, 21).

These region-specific hBOs and ‘assembloids’ can be used to model NDDs, for instance, forebrain-hBOs to model Zika-virus related microcephaly showed vulnerability of neural precursors to infection and death (22–24). Modeling brain inflammatory states in SARS-CoV-2-related encephalopathy have utilized specialized approaches, including ‘assembloids’ that incorporated non-neural ectoderm-derived cell types to like microglia, endothelia, pericytes, and CSF-producing choroid plexus (25–27). Iterations of ‘assembloids’ represent a further advance, by fusing two different organoids, thereby promoting cell mixing and potential cross-innervation. Fusing cortical and motor organoids can model cortical-spinal

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functionality (28), and fusing regionally specified hBOs van capture aspects of interneuron behavior in dorsal neocortical hBOs (29).

Patient stem cell repositories for neurodevelopmental disease

With the ability to model NDDs in hBOs, several granting agencies and family support groups have initiated efforts to generate and distribute hiPSCs from patients with specific genetic mutations, syndromes, or classes of disease. These cell lines are usually generated in a high-throughput manner using standardized methods that include checks for chromosomal and reprogramming integrity, ensuring quality. The current public libraries include the California Institute for Regenerative Medicine funded the generation of 392 hiPSC lines from patients with NDDs, now available at Fuji/Cellular Dynamics website. The Simons Foundation for Autism Research Initiative (SFARI) has funded the generation of ~150 hiPSC lines from autism patients, available at the SFARI or SAMPLED websites. NIMH repository and Genomics Resource (NRGR) offers a collection of biosamples from patients with various psychiatric conditions, including NDDs. Many of the lines document a specific molecular cause for disease, clinical phenotype, and in some cases, molecularly corrected control lines or family member control lines. If IPSC lines do not exist for a particular patient or mutation, Jackson Labs and others have precision medicine programs that can generate IPSCs on a collaborative basis, and there are several commercial options.

Recent discoveries in NDDs stem cell models

Over 50 different single gene, structural variant, mosaic and environmental NDDs causes have been evaluated in hBOs, revealing several converging findings that were not aways apparent in prior animal models (Table 1).

Mendelian Inheritance

Microcephaly.—Microcephaly is defined as having a brain head circumference less than 2 standard deviations below the mean for age, and hBOs from patients with microcephaly most often show reduced size compared with neurotypical controls. In fact, it is difficult to find reports of hBO from patients with microcephaly that lack this reduced size. This reduced size is often observed in early culture timepoints, suggesting a lesion early in embryogenesis. Several microcephaly hBO models show severely reduced size including *CDK5RAP2, CPAP, KATNB1, NARS1, PTEN,* and *WDR62* (12, 30–34). Most publications implicate defects immediately downstream of the mutant protein function, resulting in reduced neurogenesis or attenuating the neural precursor fate. For instance, *NARS1* encodes for the sole cytoplasmic asparagine tRNA transferase, required during protein translation, where biallelic loss leads to severe microcephaly. Patient cells show reduced asparagine tRNA transferase activity and impaired protein synthesis. Correlated with these findings is impaired radial glial cell proliferation in patient hBOs, evidenced by dramatically decreased MKI67 expression and cell cycle arrest (32).

Several other hBO microcephaly models also show enhanced cellular apoptosis, some correlated with increased genotoxic stress evidenced by accumulation of γH2AX, indicating DNA double-strand breaks, cleaved caspase 3, indicating apoptosis and expression of

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p53, indicating genotoxic stress. What has become clear over the past years of hBO microcephaly modeling is that many disparate pathways converge on neural precursor fate. Even for microcephaly genes encoding centrosome proteins like *MCHP1, WDR62, MCPH2, and CEP170*, among the most convergent findings are defects in cytokinesis, leading to genotoxic stress (34, 35). These insights might not have been possible without hBO modeling, because mouse models often do not show significant phenotypes.

Modeling single-gene Mendelian NDDs.—Many other Mendelian NDD conditions show robust hBO phenotypes, including defects in neuronal migration, transcriptional regulation, calcium signaling or CNS innate immunity. While mouse models of neuronal migration defects have been challenging, hBO models using patient-derived or engineered mutations, for instance with the *LIS1* gene, demonstrate critical features like impaired mitosis and spindle orientation and increased apoptosis (15). Timothy syndrome is due to genetic mutations in the L-type calcium channel gene *CACNA1C*. Mouse work suggested defects in interneuron migration, modeled in human stem cells by fusing cortical and subpallial hBOs, to study interneuron tangential migration (36). Finally, Aicardi-Goutieres syndrome type 1 is due to mutations in three-prime repair exonuclease 1 (*TREX1*), required to keep neuronal antiviral programs in check. hBOs recapitulate disease-relevant phenotypes including abundant type 1 interferon secretion and reduced hBO size triggered by LINE1 retrotransposition (37).

Modeling autism spectrum disorder.—While the molecular and cellular basis of autism shows substantial genotypic and phenotypic diversity, recent hBOs models have implicated dysregulation of the balance between excitatory and inhibitory neural cell types. These studies generally employ one of three strategies: 1] Studying hBOs from autism patient IPSC lines irrespective of the cause. 2] Studying hBOs from autism patients where a single mutated gene has been identified in the patients. 3] Introducing patient mutations into isogenic control iPSC lines to assess common mechanisms across genetic forms of the disease. The latter two approaches differ primarily in their genetic background.

Initial studies focused on the first strategy, demonstrating hBOs in idiopathic autism can show GABAergic interneuron overproduction that was traced to an accelerated cell cycle and overexpression of forebrain-specific FOXG1 transcription factor (38).

Subsequent studies focused on the second strategy, for instance, hBO characterization of one of the most common ASD genes, *CHD8*, encoding chromodomain helicase DNA-binding protein 8. hBOs show a common set of transcriptionally dysregulated genes including *TCF4* and the antisense transcript of *DLX6*, likely regulating GABAergic interneurons (39). Modeling *RAB39*-associated autism in hBOs, encoding a member of the RAS-oncogene family, show excessive growth correlated with excessive mTOR signaling (40). *ACTL6B* encodes a component of the BAF (mSWI/SNF) ATP-dependent chromatin remodeling complex, where mutations reveal a role in relieving repression of neuronal activity-dependent early-response genes (41).

Studies are emerging that seek converging evidence for the basis of autism using the third strategy. Mutations in *KMT5B*, *ARID1B*, and *CHD8* introduced into isogenic lines

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demonstrate asynchronous development of excitatory and inhibitory neurons, correlated with altered calcium dynamics and circuit activity (42).

Copy number and chromosome abnormalities

Down syndrome (DS) due to trisomy 21, is the archetypal karyotype defect with NDD. Patients show distinctive multiorgan clinical features including intellectual disability and reduced brain size. hBOs from DS patients show reduced proliferation, mildly reduced size, and lower expression of layer II and IV markers. Results indicate increased expressions of Oligo2, an early interneuron marker, as well as other interneuron lineage transcription factors. In addition, there is suppression of DSCAM and PAK1 pathways, which could be rescued pharmacologically with PAK1 agonists (43).

The most common copy number variation (CNV) in humans is at Chr22q11.2, where patients present a range of developmental and neuropsychiatric features. hBOs reveal altered resting membrane potential, leading to defects in spontaneous neuronal activity and calcium signaling, attributable to *DGCR8* that lies within the interval (44). 17q11.2 microdeletion can cause Van Asperen syndrome, displaying severe NDD. hBOs demonstrate abnormalities in neural progenitor cell proliferation and neuronal maturation, linked to reduced cytokine receptor-like factor 3 expression and impaired RhoA signaling (45). Reciprocal structural variants at Chr16q11.2 are relatively common in autism, where duplications associate with microcephaly, and deletions associate with macrocephaly. hBOs with the deletion demonstrate an excess of neurons and a depletion of progenitors, with transcriptomic and proteomic defects related to synaptic and Wnt signaling (46). Phelan-McDermid syndrome shows 22q13.3 deletion, largely attributable to haploinsufficiency for the postsynaptic density protein SHANK3. hBOs show reduced number and intensity of myelin basic protein-expressing cells (47, 48). 7q11.23 duplication, encompassing 26-28 genes, is one of the best characterized ASD-related CNVs, whereas deletion of the same interval leads to Williams-Beuren syndrome (WBS), showing hyper-sociability and language strengths. WBS hBOs capture transcriptional changes that are comparable with finding in mouse, but future work is required to uncover molecular mechanisms (49).

Somatic mosaic mutations in NDDs

Genetic mosaicism refers to mutations present in some but not all cells of an organism, often associated with specific phenotypes. Mosaicism occurring within brain progenitor cells can lead to a range of NDD conditions. Mosaic mutations are an important cause of focal brain overgrowth and neuron migration disorders, often documented by visible focal lesions seen on MRI. Examples are mosaic mutations in the mTOR pathway, with dysmorphic, disorganized neurons and glia in cortical patches linked to epileptogenesis (9, 50).

Tuberous sclerosis (TSC) is the archetypal brain mosaicism phenotype, often attributable to a ‘second hit’ leading to the loss of both copies of *TSC1* or *TSC2* genes. TSC brain tissue often shows cortical patches called ‘tubers’ containing cells carrying mutations in one or both copies of the gene. But a central question has been whether neurons or glia are the disease-relevant cell type. Some hBO modeling suggests that astrocyte dysfunction could disrupt synapses, whereas other studies implicate neuronal dysfunction

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or differentiation leading to hyper-excitability (51, 52). Recent TSC hBO data suggest that mosaic biallelic inactivation of TSC2 during neural progenitor expansion is necessary for the formation of dysplastic cells, supporting the “two-hit” model for the tuber formation (53). In contrast, recent work suggests haploinsufficiency leads to the over-proliferation of a distinctive type of neural stem cell called a caudal late interneuron progenitor (CLIP), leading to brain tumors and cortical malformations (54). Other examples of mosaic hBOs models include Rett syndrome, mutated at the X-linked gene methyl CpG binding protein 2 (MECP2). Females with mutations are mosaic for two cell populations, one wildtype and one mutant, but the proportion of cells vary by patient. Recent neurosphere studies incorporate different proportions of control and *MECP2* knockout cells, modeling natural X-inactivation variability, with results consistent with prior described defects in microRNA expression and synaptic function (55–57).

Gene-environment interactions modeled with hBOs

Gene-environment interactions refer to the alteration of phenotype resulting from differences in environment within the same genetic context. Utilizing hBOs to study the effects of environmental exposures on healthy human neurons has become a widely accepted complement to in vivo studies but combining gene and environmental factors in a single experiment is still in its infancy.

The environment can have profounds effects on brain development, function, and the epigenome, resulting in sometimes profound transcriptome alterations, for instance in the setting of smoke exposure, small molecules like pharmaceutical agents or antibiotics, organics like ethanol, hydrocarbons or insecticides, infectious agents, particulates, or heavy metals (58). hBOs may be suited for modeling environmental risks that are suspected of altering NDD risk. For instance, hypoxia and reperfusion in babies, where hBO modeling revealed the selective vulnerability of intermediate progenitors and neuronal migration (59–61). Traumatic brain injury is a major source of NDD, where hBOs captured primary pathological changes, including metabolic alterations leading to neuronal loss, and astrogliosis (62). We found that neural stem cells from patients missing adenosine monophosphate deaminase 2 (*AMPD2*) cultured in reduced levels of adenosine was uniformly lethal but tolerated in control cells (63). Organophosphates are implicated in autism risk, and when applied to *CHD8* patient hBOs, there was further reduction in CHD8 level, thus amplifying the effect of genetic risk (64).

Effects of sex and hormones on neurogenesis are understudied, often attributed to differences in sex chromosomes. However, a recent study found androgens can increase the proliferation of neurogenic pools within hBOs, mediated in part through histone deacetylase and mTOR (65). Separately, epidemiological data suggest that exposure to endocrine-disrupting chemicals (EDCs) during pregnancy could have undesirable effects on later language acquisition. EDCs like bisphenol-A, phthalates, and perfluorinated alkyl acids can disrupt hBO gene expression and neurogenesis (66). Studying such vulnerabilities may uncover mechanisms of brain resiliency.

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Strengths and weaknesses of hBO modeling

By standardizing culture conditions, 95% of hBOs can generate virtually indistinguishable compendium of cell types, following developmental trajectories comparable to the developing brain (20, 67, 68). New technologies applied to hBOs include lineage markers, retrograde synaptic tracers, and single-cell omics to study complex cellular interactions (69, 70). Several recent studies demonstrate that transcriptional signatures, cell type composition and network activity in the form of oscillatory electrographic waves observed in human fetal brain can be captured within hBOs (20, 71–73).

Weaknesses include intrinsic variability in morphology and size, which can depend to some degree on the protocol used for hBO generation (67, 74). Some of these limitations can be overcome by analysis of many hBOs per experimental condition, but still quantification challenges remain. Finally, NDDs are complex disorders, representing many hundreds of genetic causes and individual mutant alleles, likely influenced in substantive ways by genetic background, and so expectations of uniformity should be tempered (75). Therefore, careful comparisons between in vivo and in vitro models, and correlation with clinical features will remain necessary in NDD modeling.

Optimal controls in hBOs modeling

Sample-to-sample variability, potential mycoplasma contamination, culture conditions, off-target CRISPR editing, background genetic variation, and accumulated mutations during reprogramming and cell propagation, variability in reprogramming efficiency, are all potential sources of noise that prompt careful consideration of controls when evaluating NDD-relevant phenotypes. This is especially important considering how little we understand brain structure and function. Appropriate controls may include correction of the patient mutation, introduction of the patient mutation into one or more control iPSC lines, and inclusion of samples from healthy family members, or from healthy neurotypicals that are reprogrammed with the same methods. There is no perfect control for modeling, and in fact, many groups include more than one set of controls in their experiments.

Different stem cell lines can show neuronal subtype or phenotype biases, which could conflagrate results (76), and thus the requirement for controls can depend upon the effect-size of the phenotype and its proximity to the NDD-relevant disease or gene. For instance, measuring the effect of mutation in a signaling molecule on its signaling pathway is probably less subject to variability than measuring the effect on convergent phenotypes like cellular growth, differentiation or viability. Other confounding variables like mycoplasma infection in some but not all cell lines could influence phenotypes like inflammation, where there could be many factors contributing.

Balancing numbers and types of controls for given experiments to yield robust conclusions is an essential standard for all biological experiments, including hBOs. In cases when phenotypes and effect sizes are not robust, a greater number of samples and controls, including a validation cohort, is generally considered necessary. Current guidelines nearly always advise including additional control individuals and validating results with additional iPSC clones per individual whenever feasible (77).

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Patterning, gradients, and efforts to yield more uniform models

The morphology of an individual hBO is like a snowflake, meaning that no two are identical. Despite the potential for a high degree of cell type uniformity and transcriptional similarity with modern protocols, hBO variations remain due to several sources. These include cell-cell contact and cell-extracellular matrix contact within the three-dimensional structures, contributing to local morphogen, mitogen, nutrient and oxygen gradients, leading to variabilities in cell growth, differentiation, and survival (20, 67, 68). For instance, hBOs at later stages can display clusters of less healthy or necrotic cells deep within the center of the hBO, possibly resulting from lower oxygen or nutrient exposure (78), and new rosettes located towards the middle of an hBO are less likely to expand numbers of progenitors and to form layer-like structures (79).

Attempts to improve uniformity using bio-reactors and rotating platforms to increase nutrient availability, increase oxygen levels, and use microfilaments as floating scaffolds are showing promise (23, 80, 81). 'Slicing' hBOs into smaller units or culturing at the air-liquid interface show improvements in cortical layer formation and nerve tracts, and may prove to better models for certain diseases (82, 83). Improving on these results could capture more of the intricacies of human brain development.

Single rosette hBOs consist of a single luminal center with concentric rings of differentiation. Also called 'dorsal neocortical spheroids', these hBOs can be nearly morphologically identical at the cellular level, thus simplifying quantification. Generated by physically cutting a 2-dimensional sheet of neural progenitors, or using micropatterned substrates (68, 84, 85), these are much smaller than traditional multi-rosette hBOs but could be a starting point from which to introduce further complexities of brain development.

Mammalian forebrain development is regulated by morphogen gradients including sonic hedgehog (Shh), WNTs, fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), and retinoic acid (RA). These gradients help establish the dorsal-ventral and anterior-posterior and medial-lateral cell identities critical for generating varied cell types across the brain and body. Bath application of morphogens and mitogens is part of most neuroectodermal differentiation protocols. For instance, neuroectodermal EBs were first induced by dual-SMAD inhibition, Wnt inhibition can promote neuronal differentiation, while Wnt activation can promote neural crest differentiation (21, 86, 87).

Efforts to further recapitulate in vivo gradients include co-culture of hBOs with morphogen-soaked beads, morphogen-expressing cell pellets, or inducing the generation of a morphogen-expressing cell cluster, achieving some level of control over regional cell fate (88, 89). Other methods incorporate microfluidics to achieve precise gradient control, which can recapitulate aspects of neural axis rostral-caudal patterning (90). Combining single-rosette organoids with morphogens could yield further levels of control, including the specification of dorsal and ventral structures.

Micropatterned cell growth substrates containing laminin or Matrigel can promote unexpected cell-cell interactions and result in potentially useful models of NDDs. Varying the shape or size of these substrates can reveal intrinsic differentiation pathways (91).

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Micropatterned stem cell cultures exposed to Wnt and Activin can induce key hallmarks of embryogenesis, such as a 'primitive streak' and 'Spemann organizer', with spontaneously generated sharp boundary marking epithelial-to-mesenchymal transition (92), or lead to demarcated N- and E-cadherin populations, promoting neural tube-like structure that can spontaneously undergo neural tube 'closure' (93).

Integrating CNS supporting cells into hBOs

Current standard hBO protocols focused on achieving uniformity largely lack supporting cell populations of non-neuroectoderm origins, for instance, neural crest-derived pericytes and mesodermal-derived endothelial and microglia. NDDs are complex disorders, and the causes are rooted in a broad range of factors, that include contributions from supporting cells, and some recent studies have included supporting cells to hBOs. Induction of endothelial populations by expressing endothelial ETS variant 2 (ETV2) gene leads to vascularization and supports the formation of hollow blood vessels when transplanted in vivo (94, 95). Although some report that microglia can innately develop within cerebral organoids, others have added microglia derived exogenously, and with either protocol, there is evidence of microglia-mediated phagocytosis of CNS substrates that could model their homeostatic effects (96, 97). By integrating neural-crest derived pericytes into hBOs, we generated a novel Pericyte Containing Cortical Organoid (PCCO) and observed pericytes-astrocytes interactions that enhanced astrocytic maturation and neurogenesis (26).

Species comparative genomics in hBO models

One of the most exciting areas of hBOs research is in species comparative genomics. Studied incorporating non-human primates can uncover human-specific changes that may be relevant to disease susceptibility, when modeled with hBOs. Compared with chimpanzee and macaque, human hBOs development occurs at a slower pace, apparently driven by divergence in the timing of epigenetic changes in chromatin accessibility and delayed morphological state transitions (98, 99). Gene regulatory networks can show human-enriched expression in 'recently evolved' gene duplications, and in multiple regulators of PI3K-AKT-mTOR signaling (100). Correlating further hBO differences could help address whether there are disease mutations that could intriguingly produce phenotypes or susceptibilities in humans but not in other primates.

Engrafting hBOs in animal model networks

Assembloids closely modeling functional neural circuits have opened an exciting avenue for NDDs modeling (101), but a significant shortfall is the inability to assess behaviors or in vivo physiology. Can hBO human neural cells assemble into networks in rodents that participate in behaviors? Can human phenotypes be transferred to animal models by transplanting hBOs? Several groups have transplanted hBOs in mouse or rat brains to model hBO maturation and circuit assembly (102, 103). By transplanting the hBOs into the somatosensory cortex of newborn athymic rats, mature cell types are integrated into sensory and motivation-related circuits. It would be interesting to model NDDs with human cells in vivo to study behavioral phenotypes.

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Table 1.

Recent additions to the literature on hBOs modeling of neurodevelopment disorders.

Disease	Gene(s)	PMID	Finding
Germline			
Microcephaly	CDK5RAP2	23995685	Altered neurogenesis
Microcephaly	WDR62	31197141	Altered neurogenesis
Microcephaly	NARS	32788587	Increased cell death
Seckel syndrome	CPAP	26929011	Cilium disassembly
Aicardi Goutiers syndrome	TREX1	28803918	Increased cell death
Sandoff disease	HEXB	29358305	Altered metabolism
Lissencephaly	MDS	28111201, 28380362	Altered Wnt signaling and outer radial glia
Periventicular heterotopia	DCHS1/FAT1	30858616	Altered apical radial glia polarity
Timothy	CACNA1C	34990580	Calcium related hypersynchronization
Angelman	UBE3A	32916124, 31857479	Calcium related hypersynchronization
Autism	TCF4	35501322	Impaired excitability and Wnt signaling
Autism	CHD8	35385734, 28321286	Altered interneuron differentiation
Autism	AUTS2	35802027	Altered neurogenesis
Autism	ACTL6B	32312822	Altered activity-dependent transcription
Fragile X	FMRP	34413513, 33993189, 33852833	Altered neurogenesis and protein translation
Autism macrocephaly	PTEN	34461955	Altered neurogenesis
Karyotype defects			
Down syndrome	Trisomy 21	31130512	Excess interneurons
DiGeorge syndrome	22q11.2 del	32989314	Altered neural activity
16p11 deletion syndrome	16p11 del	34433918	Multiple disruptions
16p11 duplication syndrome	16p11 dup	34433918	Multiple disruptions
Van Asperen syndrome	17q11.2	34233200	Altered neural differentiation
Phelan-McDermid syndrome	22q13.3	35726031	Delayed myelination
Mosaic disease			
Tuberous sclerosis complex	TSC1/TSC2	33445520, 35084981	Altered astrocytes and cell differentiation
Tuberous sclerosis complex	TSC2	34969984	Alterned neural differentiation
Rett syndrome	MECP2	28439102	Altered neurogenesis
X-linked macrocephaly	RAB39B	32115408	Altered mTOR signaling
Nongenetic disease			
Idiopathic ASD		35618886, 35110736	Asynchronous neural development, impaired metabolism
Hypoxia encephalopathy		31417360, 31061540, 30975982, 33504071	Altered metabolism and maturation
Valproic acid exposure		35351869	Disrupted gene expression

Abbreviations: S. syndrome