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REVIEW ARTICLE

Human Brain Organoids in the Preclinical Phase of Drug Development for Migraine

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ABSTRACT

Developing drugs for brain disorders poses significant hurdles. These challenges stem from the scarcity of optimal models for preclinical drug testing and the often observed lack of translation from preclinical to human clinical trials. Further complexity arises from the specific targeting required in many brain disorders, with drug delivery often impeded by the necessity to cross the blood-brain barrier (BBB). As such, the search for novel and efficient platforms for preclinical drug development is a vibrant area of research. In acknowledgment of the limitations of animal tests - such as the lack of translation owing to species differences - and in alignment with the principles of reduction, refinement, and replacement (3Rs), the scientific community is moving towards promoting animal-free drug development plans. In this context, human brain organoids are rapidly emerging as potential alternatives to traditional methods. These early-stage in vitro models, mirroring in vivo complexities, hold great promise for preclinical drug testing for brain disorders. Stable organoid phenotypes and the uncovering of disease-specific features could soon elevate them to a valuable strategy in pharmaceutical testing for a range of brain disorders. Recent advancements in assay-ready organoid platforms and microfluidic chips present considerable potential for the application of human brain organoids in drug development. This commentary briefly discusses the generation of human brain organoids and their application in drug development with existing examples, focusing on their potential use in preclinical drug development for migraine, a prevalent, complex, and disabling brain disorder. The associated challenges and opportunities will also be outlined.

Keywords: Organoids; Brain organoids; Human brain organoids; Migraine; Drug development; Preclinical phase; Drug screening

1. Introduction

Literature concerning brain organoids, particularly human brain organoids, as novel three-dimensional (3D) in vitro models for the preclinical phase of drug development is limited[1]. Considering the attributes of human brain organoids[2,3], these structures could theoretically confer advantages, such as broader and faster drug screening, expanding avenues for exploring mechanism-based drug effects, and identifying potential side effects at the cell and organ level. Their nature allows the assessment of drug compounds' impact on brain tissue, offering a platform to test candidates for neurological or psychiatric disorders[4,5]. Additionally, they can serve as tools to evaluate whether drug candidates developed for other conditions and target organs induce any adverse effects in brain tissue, especially considering the potential blood-brain barrier crossing[5]. Presently, the primary focus lies on neurological disorders[6] and how these tools can serve as alternative instruments in drug development for such conditions. This emphasis is grounded in aging societies experiencing a rise in neurological disorders and evidence pointing to suboptimal current treatments[7,8]. The complexity of neurological disorders, coupled with a limited understanding of disease mechanisms, partly explains the challenge of identifying effective treatments[9,10]. While translational in vitro and in vivo models have aided drug development, a reliable disease model reflecting the phenotypes and mechanisms of human neurological diseases remains suboptimal, creating a bottleneck for identifying effective compounds[11]. Various animal models have been introduced to mirror aspects of brain disorders, particularly neurodegenerative diseases[12] like Alzheimer's and Parkinson's diseases. Despite advantages in certain cases, complexities in brain disorders hinder the creation of optimal animal models[11]. Ethical and economic concerns have rendered non-human primate models less favorable in drug development and rodent models have been the most popular used models. Following the principles of the 3Rs (Replacement, Reduction, Refinement) and the emergence of non-animal alternative methods, the development and introduction of reliable in vitro disease models, including organoids and micro-physiological systems, have gained extensive attention[13]. These models are anticipated to become an initial choice for drug exploration in the future. The promotion, support, and acceptance of these in vitro models by regulatory bodies, such as the FDA[13], present a significant opportunity to accelerate their implementation in the preclinical phase of drug development.

This commentary aims to briefly reflect on the opportunities and challenges of human brain organoids in unraveling brain disease mechanisms and opportunities for drug development with a focus on migraine as a brain disorder. While there is only limited literature on the use of human brain organoids for migraine[14], speculation has been drawn and presented based on existing examples from other brain disorders where human brain organoids have been examined. Scientists have so far employed self-organization or biomaterial-based techniques to construct brain-like architectures housing various tissue types, including functional neurons and glial cells[15,16]. This has led to the generation of novel in vitro brain disorder models for both neurological and psychiatric conditions[16-21]. Several challenges, however, must be overcome before fully harnessing the potential of brain organoids in brain disease comprehension and drug screening[1]. The promising outlook lies in the swift progression of technological advancements and continuous innovations[22,23].

1.1. THE GENESIS OF BRAIN ORGANOIDS

While the definition[24] of organoids may vary, these generally refer to 3D structures originating from stem cells, progenitor cells, or differentiated cells, displaying self-organization to emulate native tissue in vitro. Characterized by multiple organized cell types, organoids offer a physiologically relevant context surpassing that of conventional 2D cell cultures. Notably, their scalability makes organoids conducive to high-throughput studies, positioning them as promising systems for drug discovery. The derivation of neural progenitor cells from stem cells for brain organoids involves inducing embryonic bodies (EBs) under adherent conditions, as demonstrated by various studies[25-27]. Cortical organoids, derived from embryonic stem cells (ESCs), are generated through a serum-free culture of embryoid body-like aggregates[28]. Lancaster et al.[17] innovatively developed human-derived unguided brain organoids by embedding EBs into Matrigel droplets, resulting in organoids with heterogeneous neural identities. These methods have proven instrumental in mimicking brain development and modeling brain disorders. Utilizing a bioreactor, Qian et al.[29] crafted brain-region-specific organoids. To overcome nutrient and oxygen supply challenges in the core area during long-term culture, researchers have refined the culture method by slicing cerebral organoids[30,31]. An innovative differentiation method for human iPSCs has led to the creation of 3D brain organoids, named human oligodendrocyte spheroids, containing oligodendrocytes, neurons, and astrocytes[32,33]. Specific organoids, simulating regional features like the midbrain, hypothalamus,

blood-brain barrier, cerebellum, and spinal cord, have been developed, offering tissue-specific screening tools for investigating region-specific deficits in neurological or psychiatric diseases[34-36]. Concurrently, substantial progress has been made in enhancing the induction rate and stability of brain organoids. Notably, recent findings suggest that short-term inhibition of SMAD proteins and Wnt signaling creates an optimal condition for building a diverse cortical cell repertoire, mirroring fundamental molecular and cytoarchitectural features of cortical development[37]. This advancement in induction and long-term culture has significantly bolstered the application of brain organoids as a testing platform in drug discovery.

1.2. INTEGRATING BRAIN ORGANOIDS INTO MULTISYSTEMS FOR DISEASE MODELING AND DRUG TESTING

Interactions between various systems and brain regions play a pivotal role in the progression of neurodegenerative and psychiatric disorders. Establishing connections among brain organoids[38] and other organs holds the key to exploring therapeutic targets and advancing drug testing methodologies. One innovative strategy involves the use of assembloids[39], where different organoids are physically bound together. For example, fusing dorsal and ventral forebrain organoids to create assembloids allows for the organized arrangement of the dorsal-ventral axis in vitro, overcoming challenges related to the unidentical and random distribution of brain regions. This approach proves beneficial in analyzing complex neurodevelopmental defects involving neuronal circuits[40]. Additionally, the generation of a 3D cortex-motor assembloid, comprising the cerebral cortex, hindbrain/spinal cord, and skeletal muscle spheroids, demonstrates enhanced spontaneous muscular contraction and the formation of the motor system[41]. Cortex-ganglionic cortex assembloids mimic brain network formation and epileptiform-like activity in the brain[42].

Therapeutic targets for neurological diseases may extend beyond the brain, with organs like the liver being implicated in conditions such as Alzheimer's disease, Parkinson's disease, and depression. Organoid chips connecting different organ-specific organoids through microfluidics present a promising resolution to study these interactions[43]. Multiple organoid chips provide a platform to investigate the interplay of distant organs. Leveraging biomaterial technologies like microfluidics, 3D printing, and hydrogel shaping, brain organoids on biochips with various tissue-specific organoids replicate the communication between brain tissue and other organs. This model offers a comprehensive systematic approach to studying the development of pathologies in neurological or psychiatric diseases[44-46]. The correlation between 3D brain models created in vitro and in silico modeling has also been proposed to result in the creation of cost-effective and time-efficient in silico models, enabling the prediction of drug safety[47].

1.3. EXAMPLES OF BRAIN ORGANOIDS' APPLICATION

1.3.1. Modeling Disease Phenotypes

Brain organoids have demonstrated efficacy in modeling neurodegenerative conditions such as Alzheimer's disease and Parkinson's disease. Notably, an investigation revealed AD-like phenotypes, including amyloid beta aggregation, tau phosphorylation, and neural loss, in brain organoids exposed to patients' serum[48]. In a separate investigation employing air-liquid sliced brain organoids, those carrying the c9orf72 hexanucleotide repeat mutation exhibited associated phenotypic changes after 100 days post-slicing. These changes included the accumulation of P62, dipeptide repeats aggregation, and DNA damage—characteristic features of frontotemporal dementia[49]. The air-liquid culture method employed in this study not only enhanced neuronal survival and axonal growth during organoid induction but also proved advantageous in establishing various morphologies, such as long-range projection, growth-cone turning, and decussation.

For modeling Parkinson's disease, Jo et al.[50] presented a 3D organoid midbrain model containing functional midbrain dopaminergic neurons, capable of producing human-specific neuromelanin. These midbrain organoids exhibited mature dopaminergic functions after 65 days post-induction[50]. Upon introducing the LRRK2G2019S mutation, Kim et al.[51] induced PD-specific midbrain organoids and observed pathological changes, including the loss of dopaminergic neurons, increased apoptosis, and aggregation of α-synuclein after day 60. Smits et al.[52] compared midbrain organoids from healthy controls, patient-specific iPSCs (LRRK2G2019S), and mutation-corrected isogenic controls. They discovered the loss of PD-associated dopaminergic neurons in patient-specific organoids from day 35. Additionally, at this time point, the PD-midbrain showed an increased generation of DA progenitors, but this phenotype disappeared by day 70[52].

Brain organoids can also serve as representations of embryonic and neonatal developmental progress and are utilized as models for brain

developmental disorders[53] including autism spectrum disorders, and Down syndrome, which can be effectively represented by cerebral organoids. While much attention has been directed towards using cerebral organoids as models for psychiatric diseases, it presents distinct challenges. In organoids, where obvious neuronal deaths are absent, the initial step in organoid modeling of psychiatric diseases[54] involves identifying stable biomarkers that align with clinical or traditional animal model phenotypes. Decreased adult hippocampal neurogenesis, recognized as a biological hallmark and antidepressant target for depression, is one such marker[55]. A depressive feature in organoids can be seen as dysfunctions in radial glia-like neural stem cells, particularly related to neurogenic deregulation[56]. A study on human brain organoids indicated the involvement of serotonin receptor 6 in regulating neural progenitor self-renewal and differentiation, mimicking depressive-like behavior and adult neurogenic deficits observed in serotonin receptor 6 knockout mice[57]. Thus, neurogenesis emerges as a potential biological hallmark of depression in cerebral organoids. Considering the crucial roles of neural circuit networks in psychiatric diseases' development, the creation of fused organoids or assembloids is proposed to offer a disease model for psychiatric disorders[58-60]. Furthermore, cerebral organoids are anticipated to serve as valuable tools for unraveling the underlying biology of psychiatric diseases when they co occur with other neurological disorders. The coexistence of psychiatric conditions is frequently observed in neurological illnesses[61], such as Alzheimer's disease, Parkinson's disease, and migraine.

1.3.2. Applications in Preclinical Drug Development

In traditional drug screening, cultured cells and established rodent models like Sprague-Dawley rats or C57B/L mice have been relied upon due to their simplicity and stability. These models ensure controlled variations within groups, offering a standardized foundation. However, when compared to these models, organoids present challenges in establishing consistently stable phenotypes that effectively distinguish disease-specific features from random abnormalities. Consequently, the initial focus is on minimizing random variations during organoid generation and maintenance. This involves setting phenotypic criteria to differentiate between healthy and pathological conditions and establishing exclusive standards for abnormal organoids resulting from random factors. It is suggested that organoids can reflect the clinical heterogeneity of disease conditions and while this characteristic enhances translatability from bench to bedside, it introduces a potential downside by increasing the instability of organoids in drug screening[62]. The heterogeneity of brain organoids arises from multiple factors, including different suppliers of culture medium components, fluctuating shear forces in orbital shakers or bioreactors, and variations in the genetic backgrounds of induced pluripotent stem cells (iPSCs) from different donors. For instance, in the case of brain organoids, variability can be observed during the formation of embryoid bodies (EBs). As the culture progresses, EBs or neural spheroids exhibit different sizes due to variations in the proliferative and differentiative speeds of the progenitor cells[63]. Ensuring a stable drug screening system involving organoids necessitates minimal interference during culture and maintaining stable phenotypes between control and disease models. To address the challenge of randomization in long-term organoid culture, various approaches have been devised. Qian et al.[29] have introduced a novel apparatus called SpinΩ to control shear forces in brain-region organoids. Sliced sectional organoids or air-liquid interface brain organoids offer a strategy to enhance the long-term stability of brain organoid cultures by circumventing issues related to inadequate oxygen and nutrient supply in the core region[30,31]. Augmenting the system's efficacy in drug evaluation can be achieved by mapping the phenotypic fingerprints of brain organoids aligning with different biological events, including neurogenesis, neurodegeneration, and calcium homeostasis to mention a few. Establishing a control group for each screening batch is also pivotal in reducing organoid phenotype instability. In the screening system, control groups should consist of healthy induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs), along with isogenic control iPSCs with genetic corrections. When modeling diseases with organoids, the use of healthy iPSCs or ESCs is imperative to control the organoid generation process. Comparative analysis between healthy and isogenic controls, as demonstrated by Hinman and Burke[63], revealed that amyotrophic lateral sclerosis (ALS)-frontotemporal dementia brain organoids exhibited the aggregation of polyGA, mirroring observations in patients' brains. Moreover, the use of isogenic iPSCs helps eliminate interference arising from different genetic backgrounds.

2. Brain Organoids as A New Approach towards Unfolding Potentials in Drug Development for Migraine

Migraine, a widespread neurological disorder often underestimated in its impact, globally affects

approximately 20% of individuals[64]. Predominantly impacting women, migraines manifest as recurring intense headaches with altered sensory and motor manifestations[65]. The episodic nature of attacks reflects an evolving process driven by neural mechanisms, including neuroinflammation and central sensitization influenced by genetics and epigenetics[66]. Abnormalities in neuronal activity span various brain regions[67]. Despite progress in understanding migraine pathogenesis, treatment remains suboptimal but CGRP's involvement has recently led to the development of novel anti-migraine drugs[68]. Modeling migraines is challenging due to the disorder's complex pathogenic alterations, but animal models and human experimental pain models have been developed and offer insights[69]. Postmortem brain samples provide valuable genetic information, but studying live humans faces challenges. Migraines necessitate a holistic approach and functional brain imaging with biomarkers reveals comorbidities and highlights involved brain regions. Thalamocortical network dynamics, visual cortex abnormalities, hypothalamus, and brainstem involvement have been identified[70]. The current standard involves using a combined panel of biomarkers for a comprehensive study of migraine pathogenesis and treatment response. Utilizing human brain organoids can serve as a valuable tool in comprehending the pathogenesis of migraines and identifying novel targets for treatment[14].

2.1. FEASIBILITY OF HUMAN BRAIN ORGANOIDS AS A MODEL FOR MIGRAINE DISORDER

With the genetic and epigenetic links proposed for migraines, human brain organoids offer a promising avenue for understanding migraine pathogenesis[14]. Both guided and unguided organoids, as well as assembloids, present opportunities for modeling migraines. Unguided brain organoids, with a survival period of approximately one year, enable the study of factors influencing migraine development. This longevity makes brain organoids valuable for investigating migraine onset, pathology, and potential treatment targets. Migraine, involving various brain regions, is an ideal candidate for exploring pathogenic hypotheses. Brain organoids, resembling spheroids, offer a promising tool to identify targets and test compounds targeting molecular phases of migraines. Animal models face criticism due to the unique human nature of pain-related phenomena, making human brain organoids a more relevant organ-level model. Combining image analysis with transcription profiles allows for a comprehensive understanding of biological effects, aiding in the identification of drug targets for specific migraine features, such as abnormal calcium waves or neuronal hypersensitivity. To address the complexity of migraine, the development of region-specific organoids is essential, considering the involvement of different brain regions in various migraine phases. Utilizing systematic image analysis enables the observation and annotation of activities in various cell types, including neurons and glial dysfunction features. Integrating transcriptional profiles with image features enhances the clarity and provides supportive evidence to elucidate the mechanisms underlying different drug compounds targeting various aspects of migraine pathogenesis. Single-cell sequencing is also a promising approach to further delve into the cell-specific effects of specific drugs in organoid models, contributing to a comprehensive understanding of migraine pathophysiology.

2.2. HUMAN BRAIN ORGANOIDS IN DRUG DEVELOPMENT FOR MIGRAINE

Human brain organoids have generated significant interest, providing a dynamic and biological system for drug development, and the identification of targets for brain disorders and migraine can be an option. While brain organoids are relatively unexplored for drug screening compared to other organoids, they have shown promise in testing drugs for neurological disorders[3,62]. For instance, cerebral organoids were employed to model Creutzfeldt–Jakob Disease (CJD), successfully screening drugs such as pentosan polysulfate (PPS) for therapeutic effects[71]. Organoid models of various neurodegenerative and neurodevelopmental disorders[72], including Alzheimer's disease, Parkinson's disease, and autistic spectrum disorders, also present opportunities for drug testing. Advancements in generating CNS barrier-forming organoids (CBFOs) contribute to drug permeability testing for CNS drug development. Blood–brain barrier (BBB) organoids enable prescreening of drug candidates for permeability, aiding in identifying potential effects against neurological disorders[73]. Brain organoids have proven valuable in screening neurotoxicity at early developmental stages, and identifying drugs and chemicals with neurotoxic effects[74]. However, challenges exist, primarily related to organoid quantity, quality, efficiency, and reproducibility. Efforts are underway to address these limitations, such as integrated system-level approaches for determining drug targets and testing.

While brain organoids cannot fully replicate migraine pathogenesis, they offer the potential for drug screening and development. iPSCs from migraine patients could serve as an experimental platform, similar to applications in other disorders.

It is essential to recognize that organoid models cannot fully mimic migraine pathogenesis, yet collaborative efforts across diverse fields aim to leverage advanced technologies, research methods, and interdisciplinary expertise to explore the potential of brain organoids in migraine research and drug screening[14]. Modeling brain organoids for migraines is anticipated to involve the incorporation of various cell types, such as glia, endothelial cells, and pericytes, to explore neuroimmunological and neurovascular interactions specific to this disorder. The expectation is that robust models will enable the testing of both genetic and environmental factors contributing to pathogenesis[14]. Future developments may include the establishment of large-scale platforms for drug discovery and compound screening, expediting the development of anti-migraine drugs. The evolving technique of transplanting organoids into rodents[75] holds promise, providing circuit-wide integration for a more comprehensive understanding of migraines. The progression of human brain organoid neuroscience should be conscientiously aligned with legal and neuroethical considerations[76] to ensure the code of conduct[77] and ethical generation and application of these intricate entities. The central focus of organoid intelligence (OI) research[23], an emerging cientific discipline, has also come to the forefront in terms of ethical considerations. Biocomputing systems rooted in OI have been suggested to facilitate accelerated decision-making, ongoing learning, and enhanced data efficiency.

Figure 1. Presents various potentials for the use of human brain organoids, from understanding pathogenesis to testing drugs and exploring new potentials for targeting migraine[14].

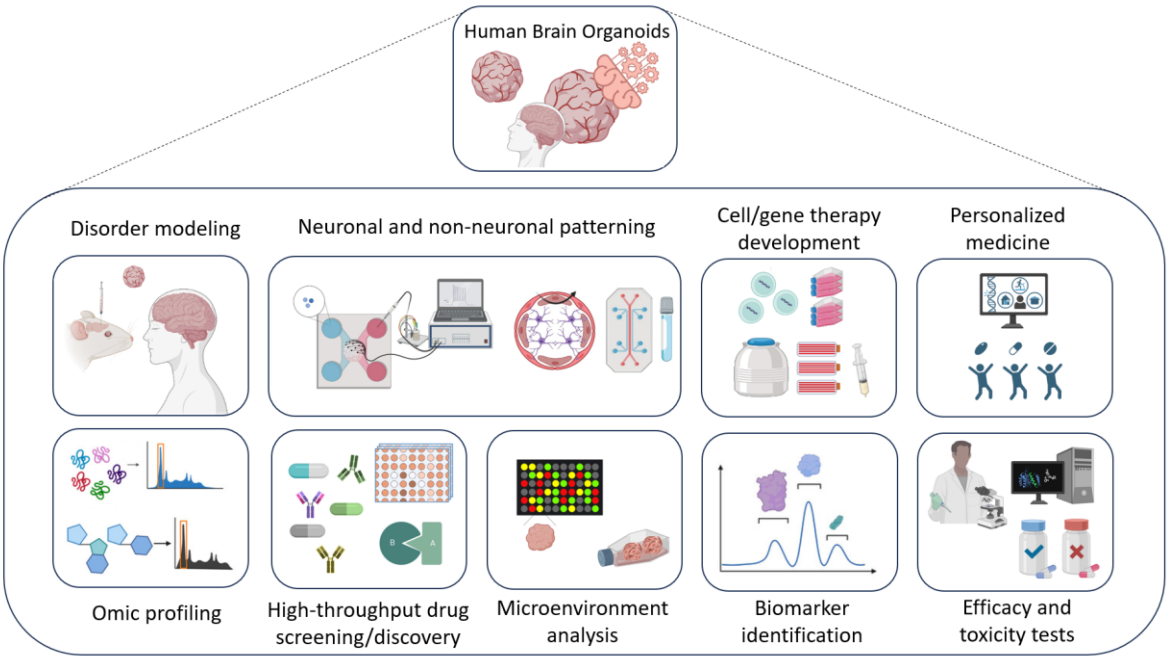

Figure 1. Exploring the Multifaceted Potential of Human Brain Organoids: From Unraveling Migraine Pathogenesis to Drug Testing and Discovery of Novel Therapeutic Targets. *Created with BioRender.com*

3. Limitations of Human Brain Organoids

While human brain organoids have significantly advanced disease modeling and potentiated drug discovery opportunities, there remains a notable need for improvement, specifically in bridging the gap between organoids and their endogenous counterparts. Despite the potential of human brain organoids, several challenges persist in current organoid systems, which need to be addressed to enhance their utility. Notably, a lack of standardization in organoid research introduces sample heterogeneity, as diverse protocols are used for various organoids. Creating identical organoids is challenging, requiring thoughtful assay design for drug screening. This lack of uniformity raises concerns about the reproducibility and comparability of research findings across different studies. Ideally, the best model systems should replicate intrinsic spatiotemporal patterning processes, display characteristic markers and phenotypes, achieve adequate maturity, and encompass key cell-to-cell interactions, an appropriate microenvironment, and suitable mechanical properties. They should mimic human pathologies faithfully, be scalable for high-

throughput screening, and exhibit features such as dynamic flow to enhance their relevance. Despite these limitations, brain organoids hold promise as valuable biological in vitro models for disease modeling and drug screening, provided that ongoing efforts address these challenges to improve their accuracy and reliability.

These general limitations are valid in the context of neurological disorders and migraine. However, human brain organoids represent a promising avenue for advancing our understanding of migraine pathogenesis and identifying new treatment targets. Their potential lies in replicating complex neural mechanisms associated with migraines, allowing researchers to study the intricate interplay of genetic, epigenetic, and environmental factors contributing to the disorder. Additionally, human brain organoids can offer a unique in vitro platform for drug screening, potentially enabling the development of targeted and more effective migraine treatments. Furthermore, integrated organoids and advanced micro-physiological systems have the potential to theoretically explore and target abnormalities in neuronal, vascular, and brain connectivity in an in vitro multisystem-integrated platform.

4. Conclusions

To improve drug exploration efficiency and identify effective compounds in the future, in vitro disease models with distinct phenotypes are crucial. Brain organoids offer a 3D human-derived in vitro brain model, incorporating diverse cell types and morphologies akin to the in vivo environment. As methods for stabilizing organoid phenotypes advance and disease-specific features are uncovered, brain organoids are expected to emerge as a primary strategy in modernized preclinical drug testing for various brain disorders, including migraine. While limited studies currently showcase brain organoids as drug screening models, their potential is anticipated to grow as challenges related to the phenotypic landscape, culture conditions, and evaluation standards are addressed. The application of 3D human-derived organoids, mimicking organogenesis, holds promise for drug testing in complex neurological or psychiatric disorders. Leveraging conditional medium from organ-specific organoids or organoid chips to connect brain organoids with others via microfluidics is considered advantageous. A library of drug compounds for brain organoid screening, guided by a target disease approach, is expected to be developed. Brain organoids are proposed to serve as cutting-edge tools for studying and identifying novel targets in neurological or psychiatric diseases, offering screening platforms for drug candidates in the preclinical phase. Although the precise mimicry of migraine phenotypes remains under construction, ongoing progress suggests promising applications. The advent of patient-derived organoids in cancer biology has demonstrated their utility as in vitro avatars for various indications, including drug screening platforms. Companies have introduced assay-ready organoid platforms, enabling short timelines, repeated assays from a single batch, high throughput, and large panel screens. These technologies offer predictive and reproducible organoid drug testing, facilitating and accelerating preclinical drug development.

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