Human brain organoids combined with CRISPR technology to gain insight into neurological diseases

He Yin *

School of Life Science, Shanxi University, Taiyuan, China

* Corresponding author: 202101402141@email.sxu.edu.cn

Abstract. As the most complex organ of human beings, the brain's functions cover various high-level intellectual activities such as cognition, language and thinking. These functions are closely related to the developmental processes in the cerebral cortex granted the composition structure of the nervous system in the cortex. Therefore, scientists have long been exploring the developmental processes of the cerebral cortex and the constituent structures of the nervous system, the purpose of the system is to deepen comprehend how the brain works. The study of neurological diseases by improving three-dimensional brain models is a hot issue at present, because the brain is very complex and fragile, and many therapies are weak and still theoretical. Brain organoids are a kind of three-dimensional tissue culture derived from human pluripotent stem cells (hPSCs), which can simulate the type composition, spatial structure, physiological function and other characteristics of human brain cells by culture in vitro. The use of brain organoid technology can simulate the development process of the brain in vitro, providing an unprecedented opportunity to study the occurrence of brain function and nervous system diseases, and combining CRISPR technology for screening, so that it can be further applied efficiently. This research will summarize the development process, progress and deficiency of brain organoids and the research situation of neurological diseases, and make a summary of the current development and prospect of the future development.

Keywords: CRISPR technology, Brain organoids, Neurological disorders, Screen.

1. Introduction

Neurological diseases are an extensive of diseases affecting the structure and function of the nervous system, including a variety of different conditions, such as Alzheimer's disease (AD), Parkinson's disease (PD), autism spectrum disorders (ASDs) and Huntington disease (HD). Compared to other species, the uniqueness of the human brain is evident, not only in its structure and function, but also in the possible emergence of brain diseases. Because of this uniqueness, the limitations of using animal models to study human brain diseases cannot be ignored. It is worth noting that genes affected by spontaneous mutations usually express or code for chromatin remodeling proteins during embryonic development. Additionally, the altered genes enhanced coding products related to transcriptional regulation and synapse development. Exome sequencing can be used to identify genes linked to neuropsychiatric illnesses. While multiple clinical studies have shown a causal relationship between multiple gene mutations and autism, scientists still do not fully understand how these genetic mutations lead to brain defects. We need a more human brain model to study.

The first 3D human brain organ generation was reported in 2013 [1]. In this study, the research team first assembled ESCs from human embryonic tissue and iPSCs from adult tissue into embryoid bodies. They then placed these embryoids in a neuro-induction medium to induce neuroectoderm formation. Once the neuroectoderm formed, they transferred the embryoid bodies into the stromal gel to expand the neuroepithelium. Finally, they grew these tissues in a rotating bioreactor to enhance nutrient absorption and promote further maturation and preservation of the tissues [1]. Brain organoids are differentiated from human pluripotent stem cells in three-dimensional culture and can self-assemble to form a characteristic ventricular zone (VZ) structure, which consists of a variety of neurons including deep neurons and shallow neurons, as well as astrocytes, neural progenitors, radial glia and oligodendrocytes. It is similar to the cell composition, tissue structure and developmental trajectory of human brain, and can simulate the structure and electrophysiological characteristics of

human brain during embryonic development [1]. Rebuilding the brain-mimicking microenvironment via engineering has made it easier to create an enduring brain organoid grow platform, which has aided in the efficient modeling and medication development of disorders of the human brain [2]. Therefore, this research will analyze the research progress of brain like organs and point out the existing shortcomings. And this research will also analyze the current research status of neurological diseases and discuss the development trends of the disease.

2. Brain organoid models

Segmented brain organoids rely on exogenous inducible factors that precisely control the composition and timing, and cell differentiation generates brain organoids with molecular, cellular, and anatomical characteristics similar to specific brain regions, with higher controllability and consistency, which is suitable for the study of neurotoxic substances with clear target cells. With the in-depth study on the development of brain organoids, some new methods have been used to improve the differentiation level of brain organoids. These new methods not only improve the development degree and maturity of brain organoids, but also provide a more accurate and reliable model for the study of nervous system diseases. By combining the brain organoids with CRISPR technology to screen the genes of autistic patients, brain organoids have revealed the enormous potential and complexity of brain function in neuropsychiatric disorders, which makes them an ideal model for studying neuropsychiatric diseases. Organoids these tiny groups of cells grown outside the body that mimic the differentiation of tissues-give us an individual perspective to study these questions, but only if we can record their electrical activity for weeks or months as they grow. As shown in Fig. 1, a complex electrode basket can be used that supports long-term growth and electrical recording of brain organoids [3].

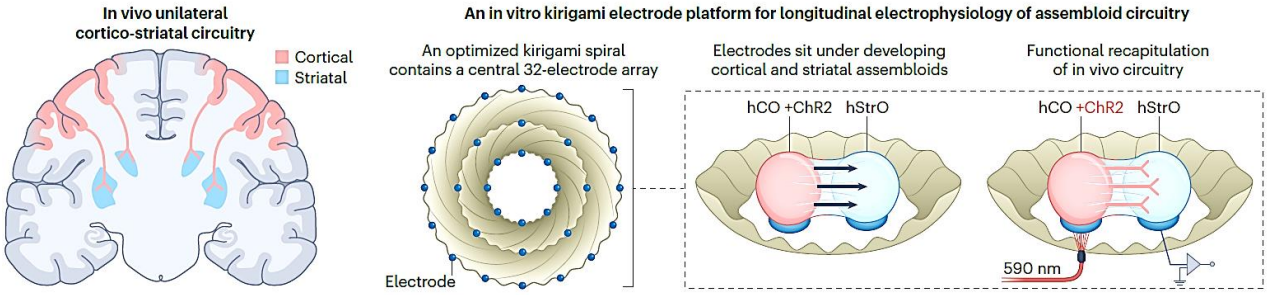


Figure 1. A developed electrode platform for longitudinal electrophysiology in assembly circuits [3].

2.1. Development of improved human brain organoid culture

Human pluripotent stem cell-derived brain organoids offer useful for in vitro study algorithms to broadly grasp human brain development and neurological disorders. Several bioreactors, biomaterials, devices, and genetically modified bioengineering strategies have been shown to overcome these limitations of current brain organoid culture. For example, synthetic polymer microfilaments guide self-organization by promoting the extension of neuroepithelium, thus promoting the formation of neuroectoderm and the development of cortex [4]. In another study, small rotating bioreactors were used to improve the dynamic culture of brain organoids, resulting in a more robust disease model of Zika virus infection. In order to increase oxygen supply, air-liquid interface culture was applied to brain organoids, resulting in improved survival and morphology, and extensive axon growth [5].

2.2. Brain extracellular matrix (BEM)

BEM can offer a greater variety of biological elements that impact the formation of organoids in the human brain. For example, upregulation of neurocan and tenascin-C was observed in a mouse model of temporal lobe epilepsy, while major ECMs involved in tissue remodeling, such as

glycoproteins, laminin, and fibronectin, did not change significantly. BEM organoids may have more NPs than Mat organoids, while also undergoing enhanced neurogenesis, which can be seen in the up-regulated expression of progenitor cell markers (SOX2) and neuron markers (TUBB3). This may be because the proliferation and differentiation of NPs occur simultaneously in the early stages of brain organoid development (~30 days) [2]. During brain development, the rate of proliferation and neurogenesis is constantly changing, the balance of an individual is believed to play a significant role in determining the size of their brain. During early development, radial glial cells (RGCs) are a major reservoir of progenitor cells that give rise to neurons and glial cells and serve as axes that guide neuronal migration. The researchers compared the transcription profiles of brain organoids with data collected from specific areas of the fetal and adult brains; A Pearson correlation analysis of three different parts of the frontal lobe of the human brain and five regions of the brain, including the cerebellar cortex, the hippocampus, the cerebellum, the striatum, and the medial dorsal nucleus of the thalamus, showed that two brain organoid groups were generally more closely related to the fetal brain [2]. Similarly, organoids associated with the other five brain regions showed a stronger correlation in gene expression patterns with those of the fetal brain [2]. Of particular interest, specific cell populations in BEM organoids showed responsiveness to gamma-aminobutyric acid (GABA) treatment [6].

2.3. Deficiencies

Although brain organoid technology shows great potential, however, it still faces several challenges. One is that it is possible to simulate the later stages of neurogenesis after 6-9 months of in vitro culture. This long cultivation process poses certain limitations to the efficiency and practicability of the research. Human brain organoids still need to be further improved in the development of neurons, the growth and maturation of structures, and the production of better electrophysiological functions. Brain organoids grown in petri dishes or test tubes lack a true vascular system, resulting in an inability to efficiently transfer nutrients to the interior of their three-dimensional structures, thus significantly limiting their structure and function. This phenomenon constitutes a significant technical obstacle to the development and maturation of brain organoids [7]. This reduces the survival time and complexity of organoids because cells cannot divide many times to increase their number or diversify cell types. Although some researchers have tried to overcome these limitations through synchronous implantation of blood vessel tissue, this approach has not fully simulated the microenvironment of real brain cells, and therefore has not fully solved the problem of efficient delivery of nutrients within the three-dimensional structure of brain organoids. The Gage lab sought to replicate a more supportive physiological environment by taking organoids based on human stem cells all the way to a vasculose-rich region of the mouse brain, where transplanted human organs were integrated into the host environment, forming neurons and neuron-supporting cells called astrocytes, and being investigated by immune cells. They also saw blood vessels flowing through them-a first for organoids [7]. At the same time, it should be noted that the factors affecting brain development are complex, and the regulation of some influential factors is lacking in the process of brain organoid culture, especially the regulation of neurodevelopment by other organ systems except the central nervous system [8].

3. Autism spectrum disorders (ASDs)

ASDs and other neurodevelopmental disorders (NDDs) are closely related to the abnormal development of cortical interneurons. This discovery provides new insights into the pathogenesis of these complex diseases. But which of these NDDs genes influence the production and migration of interneurons, and how they mediate these effects, remains unclear [7]. Deficits in the development and function of GABAergic (gamma-aminobutyric acid release) interneurons have been linked to ASDs and NDDs [7]. ASDs are the group of neurodevelopmental disorders characterized by significant impairments in social interaction and communication, often accompanied by stereotypical

behavior patterns and repetitive interests. The term "ASDs" includes autism, generalized developmental disorders not otherwise defined, and Asperger's syndrome. ASDs present a heterogeneous cause, and there is no clear medical test or treatment. ASDs affects about 1 in 150 children, with males affected three to four times more often than females. ASDs are usually diagnosed between the ages of 3 and 6, however, there is growing evidence that some children may exhibit symptoms associated with ASDs before the age of 2, potentially leading to diagnosis at an earlier age. After extensive research, genetic variants associated with ASDs have been identified in hundreds of different genes. Most of these variants are rare types and cover a broad spectrum of mutations, from small changes in a single base pair to deletions or duplications involving thousands or even millions of base pairs [9].

The new name is autism spectrum disorder (ASD), which includes the original autism or autistic disorder, Asperger syndrome and childhood disintegrative disorder and Pervasive developmental disorder not otherwise specified (PDD-NOS). That is, in the past, we often hear, and the most commonly compared autism, Asperger's disease, will be collectively referred to as the ASDs. For example, the various colors in the spectrum, each color symbolizes the possible symptoms of autism or the characteristics that can accompany it, such as language ability and intelligence status, and the dark colors represent the severity of symptoms, each autism, is a unique spectrum, such a diagnosis and classification is more appropriate to the condition of autism, and can better reflect the advantages and disadvantages of autism.

To open the "black box" of autism research, a new technique has been used to screen key transcriptional regulatory genes for autism [10]. In the newly developed system called "CHOOSE" (CRISPR-Human organoid scRNA sequencing), each cell in the brain organoid carries up to one specific gene mutation. Researchers can trace the effects of each mutation at the single-cell level and map the developmental trajectory of each cell [10]. Brain samples of patients were collected for in vitro culture of brain organoids, and gene sequences that may cause diseases were screened out, which greatly deepened the cognition of neurological diseases and gained new insights into neurological diseases such as autism.

P-cresol has the property of crossing the blood-brain barrier and consumes reduced glutathione in the brain. Abnormalities in the brain's prefrontal cortex, which regulates emotional, cognitive and decision-making functions, have been linked to autism. Importantly, when p-cresol is metabolized, it accumulates in the prefrontal cortex. This process leads to depletion of reduced glutathione, which in turn activates microglia, triggering oxidative stress and neuroinflammation. These responses eventually lead to damage and apoptosis of neurons in the prefrontal cortex, which induces the symptoms of autism. Therefore, to gut microbiota metabolites are strongly associated with autism spectrum disorders.

CRISPR-Cas9 can be used to produce such genetic diseases and control organoids, this allows for an in-depth study of a single mutation during brain development, which is an improvement on using unaffected family members as controls [10]. Although off-target effects can still introduce unwanted mutations. The iPSCs-derived brain organoids are the model of choice for ASDs research with few ethical flaws, brain organoids are an ideal platform for exploring pathogenic genetic variation [11]. In addition, it is expected to improve the accuracy and fidelity of the simulation of ASDs-specific human brain development using two-dimensional cultures and animal models. Adding CRISPR-Cas9 to create isogenic controls makes this model even more powerful by greatly reducing genetic background differences.

4. Conclusion

Unlike many other brain disorders, ASDs have not yet found a clear unifying mechanism at the molecular, cytological, or systems level. It is not yet clear whether autism is caused by mutations in some common molecular pathways or a range of different types of institutional disorders. ASDs appear to be caused by factors that affect the development of many or all functional brain systems. It

is important to note that the mechanisms of disorders such as autism are closely linked to the timing of brain development, particularly in the early stages after pregnancy. Studies of neuroanatomy and teratology have shown that the occurrence of these diseases is closely related to changes in early brain development. This anomaly indicates that the brain is clearly influenced by environmental factors and triggers a chain of disease-causing events. How the complex neural circuits of the human brain form and the mechanisms of maturation during prenatal development remain largely an unsolved mystery. In children with autism, the brain tends to grow faster than normal after birth, and then grow normally or relatively slowly during childhood. However, it is unclear whether this excessive proliferation in the early stages in all children with autism. This appears to have the most prominent effect on brain regions that develop higher cognitive specialization.

It is estimated that under this method, the disease development process that may be caused by the pathogenic gene sequence in the patient's brain can be simulated, and whether the development of the brain organoids can be normalized after the development of the human brain embryo or the knockout of the pathogenic gene can be simulated, and the abnormal changes and mechanisms of the brain under the disease state can be revealed. By simulating genetic mutations and phenotypic characteristics of patients, patients can be provided with customized treatment options and drug development can be accelerated.

References

[1] Lancaster M A, Renner M, Martin C A, et al. Cerebral organoids model human brain development and microcephaly. Nature, 2013, 501 (7467): 373 - 379.

[2] Cho A N, Jin Y, A Y, et al. Microfluidic device with brain extracellular matrix promotes structural and functional maturation of human brain organoids. Nature communications, 2021, 12 (1): 4730.

[3] Brandt J N, Rajasethupathy P. Eavesdropping on brain organoids. Nature Biotechnology, 2024: 1 - 2.

[4] Lancaster M A, Corsini N S, Wolfinger S, et al. Guided self-organization and cortical plate formation in human brain organoids. Nature biotechnology, 2017, 35 (7): 659 - 666.

[5] Giandomenico S L, Mierau S B, Gibbons G M, et al. Cerebral organoids at the air–liquid interface generate diverse nerve tracts with functional output. Nature neuroscience, 2019, 22 (4): 669 - 679.

[6] Muguruma K, Nishiyama A, Kawakami H, et al. Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. Cell reports, 2015, 10 (4): 537 - 550.

[7] Mansour A F, Gonçalves J T, Bloyd C W, et al. An in vivo model of functional and vascularized human brain organoids. Nature biotechnology, 2018, 36 (5): 432 - 441.

[8] Wang S, Harvey L, Martin R, et al. Targeting the gut microbiota to influence brain development and function in early life. Neuroscience & Biobehavioral Reviews, 2018, 95: 191 - 201.

[9] Vorstman J A S, Parr J R, Moreno-De-Luca D, et al. Autism genetics: opportunities and challenges for clinical translation. Nature Reviews Genetics, 2017, 18 (6): 362 - 376.

[10] Li C, Fleck J S, Martins-Costa C, et al. Single-cell brain organoid screening identifies developmental defects in autism. Nature, 2023, 621 (7978): 373 - 380.

[11] Kawatani K, Nambara T, Nawa N, et al. A human isogenic iPSC-derived cell line panel identifies major regulators of aberrant astrocyte proliferation in Down syndrome. Communications Biology, 2021, 4 (1): 730.