

Application of neural organoids in studying neurodegenerative diseases

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Abstract. Neurodegenerative diseases are among the top causes of mortality and the aversion of DALY's (Disability-Adjusted Life Years) worldwide. Many attempts have been made to develop therapeutics to alleviate disease symptoms without much success. The preclinical models utilized in therapeutic testing are often inaccurate and cannot precisely translate into clinical studies. The introduction of neural organoids, a three-dimensional model grown from human-originated stem cells, was able to revolutionize the field of neurological drug development. Using induced pluripotent stem cell (iPSC), scientists are able to restore adult cells' pluripotency and cultivate them into region specific brain organoids using a combination of growth factors, agonists, and inhibitors. These models have proven valuable in drug screen for myriad neurodegenerative disorders. To model such diseases, iPSCs are generated from patients with the respective diseases, and then cultivated in an environment that mimics the disease environment. For example, for Parkinson's disease, Wnt pathway inhibitors and the Sonic hedgehog agonist are used to induce midbrain neural progenitor cells from patients with risk factors. Despite neural organoids' wide usage in screening for neurodegenerative disorders and drug testing, neural organoids present several limitations in their function, including a lack of complexity equivalent to that of the brain. This paper will discuss neural organoid technology and provide basic insight to its usage in drug screening and the field of neuroscience.

Keywords: Neural Organoid, Neurodegenerative Disease, Induced Pluripotent Stem Cells.

1. Introduction

Neurodegenerative diseases are a prominent cause of DALYs and mortality globally [1]. Despite significant progress made in the field of neuroscience, we still face a huge gap between increasing medical needs and the lack of disease-modifying therapy. Traditional approaches to studying brain diseases primarily relied on animal disease models, post-mortem human brain samples, and two-dimensional cell cultures. However, these methods are limited in their capability of replicating the complex three-dimensional architecture and multiple functional areas of the human brain. This limitation has impeded the translation of basic research findings into effective treatments for neurological disorders.

Neural organoids are a promising technology capable of recapitulating processes in the brain. Neural organoids, also known as brain organoids or cerebral organoids, represent 3D structures of neural progenitor cells that self-organize into intricate structures reminiscent of various regions of the

developing brain. These miniaturized replicas, cultivated from human pluripotent stem cells (hPSCs), hold potential to provide explanations to processes such as neural circuit formation, cellular diversity, and the perturbations underlying neurological disorders [2]. Using the method of induced pluripotent stem cells (iPSC), adult tissue pluripotency may be restored, which allows them to be cultivated into organoid structures. With technological advancements, such structures could more accurately recapitulate the complicated brain functions and operating systems and hold the promise to identify causes and characteristics of prominent neurological disorders, including AD and PD. iPSCs can be derived from the fibroblast of subjects carrying certain genetic mutations that predispose him/her to a particular neurological disorder. Using the CRISPR technique, the genetic mutation could be corrected in certain neural cell types, i.e., neurons or astrocytes. By comparing the neural organoids grown from these iPSCs, the researchers could determine contributions of certain genes in different cell types to disease progression in a controlled environment. Furthermore, neural organoids can be employed for more effective drug screening, as well as the effects of certain drugs on brain activity. With simplicity, neural organoids are great tools for study.

This review focuses on the development of neural organoid technology and its use in research and drug development. This review will first introduce the assembly process of neural organoids, and then provide an overview of its usage in studying common neurodegenerative diseases such as AD and PD. Finally, this review will discuss the extent to which neural organoids have successfully modeled brain structures and served as a competent model for investigating neurological diseases and identifying possible future directions and improvements of neural organoids.

2. Assembly of Neural Organoids

2.1. Induced Pluripotent Stem Cell Technology

Introduced in 2006, iPS technology relies on four genes to restore cell pluripotency, being Myc, Oct3/4, Sox2, and Klf4 (named “Yamanaka factors”), which are either associated with the maintaining of pluripotency or discovered to be capable of producing iPS [3]. Using a retroviral or lentiviral system, these four genes can be introduced into cells and facilitate reprogramming based on each gene’s functions. The implementation of iPSC technology in the cultivation of neural organoids solves ethical issues and greatly decreases the amount of resources required for sample collection, as opposed to directly collecting embryonic stem cells. Using iPS technology, adult epithelial cells, fibroblasts, or other cells can be converted into pluripotent forms. When cells are collected from patients, whom may or may not carry genetic mutations, certain predisposing genetic mutations, the particular genetic, epigenetic, or patient-specific factors could be studied in the generated neural organoids. Human iPSCs have widely been used in basic research and drug development [4] and are an essential breakthrough for the cultivation of neural organoids. Currently, there are both non-profit iPSC consortium and commercial sectors generating iPSC lines on various genetic/disease backgrounds that are accessible to both academia and industry.

2.2. Neural Organoid Cultivation

Although various neural organoid culture protocols were developed by different laboratories, the successful methods were based on accumulated knowledge from studying neural development and experiences of directed 2D cultures. Without going into details, the common theme of these methods is described below.

When neural spheroids or embryoid bodies (EBs) are first generated from iPSCs, iPS cell medium supplemented with Dorsomorphin, which inhibits the bone morphogenetic protein (BMP) pathway, and SB-431532, which inhibits transforming growth factor- β (TGF β) signaling and thus suppresses the differentiation towards mesoderm and endoderm cells, is used to cultivate the cells and induce neural progenitor cell (NPC) generation [5]. After changing the cell medium on the 5th-10th day, the iPS medium is replaced with neural growth medium supplemented with various growth factors, including EGF and FGF-2, which induce cell proliferation and assist early-stage organoid development; BDNF,

which promotes neurogenesis and neuronal plasticity [6]; and GDNF and NT3, which improve the neural cell maturation and survival. Differential combinations of these growth factors lead to the development of region-specific neural organoids.

3. Application of Neural Organoids in Studying Neurodegenerative disorders

3.1. Alzheimer's Disease (AD)

AD is a major form of dementia. One major characteristic of AD is progressive memory loss, which could be accompanied by age-related brain atrophy and the leakage of the BBB. Microscopically, amyloid-beta plaques and tau tangles are evident in the brain section of AD patients. There are familial and sporadic forms of AD. The former is associated with a single gene mutation that results in impaired processing and the accumulation of amyloid-beta. The three well-recognized familial Alzheimer's disease (fAD) genes are APP, PSEN1, and PSEN2, whose mutations may cause early-onset familial AD [7]. Animal models and human iPSC carrying these mutations have been widely used to study the mechanism and progression of AD.

The sporadic form accounts for more than 95% of Alzheimer's disease. In this case, the combination of predisposing genetic factors and environmental risk factors work together for the manifestation of disease. One prominent genetic contributor to sporadic Alzheimer's disease (sAD) is ApoE4 alleles. To model Alzheimer's disease using brain organoids, iPSCs are generated from AD patients with or without predisposing genetic factors (mutations or diseases associated alleles or variants) and cultivated to form organoids. As one example to bring in environmental risk factors, human serum was added to the neural organoids to mimic the breakdown of the BBB. After contact with serum, organoids are expected to experience pathological conditions that may result in the accumulation of amyloid-beta plaques and tau tangles, a characteristic of AD-influenced brain cells [8].

3.2. Parkinson's Disease (PD)

PD is a frequently diagnosed neurodegenerative disorder. The major symptoms include unintended movements, difficulties in walking and talking, slowness, and stiffness, which are the result of gradual damage of dopaminergic neurons in the substantia nigra. At a cellular level, Lewy bodies with alpha-synuclein aggregation are characteristic pathology features of PD. Similar to AD, there are inherited and sporadic forms of Parkinson's Disease. About 20 disease-causing genes have been identified through the years [9]. Among these genes, some are involved in processing alpha-synuclein proteins, such as SNCA, GBA, and PARK5, some are involved in mitochondria functions, like PINK1, and some are involved in multiple cellular functions that remain to be fully understood, such as LRRK2.

To properly model PD, iPSCs were generated from the fibroblasts of PD patients carrying risk genes. Optimized protocol was used to generate midbrain organoids. Besides inhibitors of BMP and TGF β pathways, Wnt pathway inhibitor (i.e. CHIR-99021) and Sonic hedgehog agonist (SHH) are often used to induce the midbrain neural progenitor cells. It is important to confirm the presence of dopaminergic neurons by marker staining (e.g. FOXA2, TH) and functional analyses (Ca²⁺ flux and electrode recording).

4. Perspectives

Neural organoids are a promising technology that can be utilized to test potential therapies for many neurodegenerative diseases and potentially uncover a rational explanation as to how the brain functions. These organoids have emerged as a transformative advancement in the field of neuroscience, offering an innovative approach to studying complex neural processes in vitro. Using a three-dimensional model cultivated from iPSCs, these organoids are useful for drug testing and investigating brain processes. These organoids can be modified through genetic methods (CRISPR, knockdown or overexpression) to understand the contribution of a particular gene. The culture conditions could also be modified to mimic brain environments of certain disorders allowing them to better model such diseases.

Neural organoids can also be used in modeling stroke, which is prevalent in the U.S. and many other regions worldwide. To resemble the ischemia caused by fatty buildups blocking blood vessels to the brain, the standard oxygen can be replaced with nitrogen in the medium. Further investigations have also been made regarding possible genetic causes of stroke, which can also apply to other neurodevelopmental or neurodegenerative diseases [10].

Though neural organoids provide a more accurate three-dimensional model compared to traditional animal brains or two-dimensional models, they are yet to be able to fully replicate the structures of the human brain. While they provide a simplified representation of brain complexity, they lack the full spectrum of cell types and intricate neural circuitry found *in vivo*. Moreover, since many neural organoids are only cultivated less than 100 days, they may not be capable of modeling the fully developed adult brains and may only be able to provide insight into early stages of brain development.

Furthermore, neural organoids lack vascular networks and complete structures of the human brain, which may limit their ability to model certain stages of disease progression or development. In addition, neural organoids are unable to produce microglia, which are immune cells in the central nervous system that may influence brain development and its respective homeostasis [11] and could contribute to disease progression. As a result, these organoids may present certain liabilities when being used to screen drugs for certain diseases or model certain functioning processes of the brain. In summary, while neural organoids are a promising technology in the field of neuroscience, many improvements can be implemented to the model to allow them to function more precisely.

Neural organoids can be improved in many respective methods. One area of concern is how these models can simulate the entire brain environment compared to one single area of the brain. Current methods can only allow iPSCs to be cultivated into one designated region of the human brain, which may not accurately model specific brain responses or disease-causing genes because the human brain operates contemporaneously. A possible solution is to assemble two or more region-specific organoids together. For example, assembly the cortical organoids with subpallial organoids generate forebrain organoids, which could be useful to study the migration of GABAergic neurons and the motor and emotional responses.

The induction of cellular aging techniques could potentially allow organoid models to better resemble the brain environments of AD patients. Current methods include induced cellular senescence, which may be able to produce cellular aging; however, its effect on iPSCs is yet to be tested, and its potential side effects are yet to be discovered. Cellular senescence is heavily tied to aging and can induce metabolic disorders [12], similar to neurons under AD influence, which are largely affected by cellular dysfunction and structural damage. However, ensuring that only specific cell types within the organoid undergo senescence could be challenging, and consistent changes created from senescent technology may be hard to produce as senescent cells are often characterized by heterogeneity.

5. Conclusion

The incorporation of organoid technology into brain research is on a major scale; however, neural organoids cannot account for many aspects of the brain environment due to its current limitations. Neural organoids have increased clarity on the understanding of common neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease, allowing us to identify characteristics of these diseases and confirm causation relationships between certain gene strains and the disease. Overall, during the past decade, major improvements have been made in neural organoid culture to model the brain function and study the disease progression. As time progresses, more developments will be made to improve the functions of organoids and increase its consistency and reproducibility. New methods such as 3D models, which will provide better imaging of the brain and respective conclusions drawn from new discoveries, and improved pluripotent stem cell technology all have the potential to improve the quality of organoid discoveries. In summary, neural organoids remain a promising technology that will continue to revolutionize the healthcare industry and the whole field of science.

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