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Modeling neurological diseases using iPSC-derived neural cells:

iPSC modeling of neurological diseases

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Abstract

Developing efficient models for neurological diseases enables us to uncover disease mechanisms and develop therapeutic strategies to treat them. Discovery of reprogramming somatic cells to induced pluripotent stem cells (iPSCs) has revolutionized the way of modeling human diseases, especially neurological diseases. Currently almost all types of neural cells, including but not limited to neural stem cells, neurons, astrocytes, oligodendrocytes and microglia, can be derived from iPSCs following developmental principles. These iPSC-derived neural cells provide valuable tools for studying neurological disease mechanisms, developing potential therapies, and deepening our understanding of the nervous system.

Keywords

iPSC; neurological diseases; disease modeling

Introductions

iPSCs are a type of cells that can be generated by reprogramming somatic cells using four transcriptional factors, OCT4, SOX2, KLF4, and MYC (Okita, et al., 2007, Takahashi, et al., 2007). iPSCs possess pluripotency like embryonic stem cells (ESCs) and can be induced to all cell types within human body under appropriate culture conditions. Since the establishment of this technique, iPSCs have been applied to studying a variety of diseases and generated the concept of “disease in a dish”, meaning modeling disease phenotypes using iPSC-derived relevant cell types in a tissue culture dish (Shi, et al., 2017). iPSC-based disease models have also been used for drug screening, aiming at alleviation of disease phenotypes by targeting specific molecular mechanisms identified using the models.

iPSC-based disease modeling starts from generation of iPSCs from somatic cells, e.g. fibroblast (Takahashi, Tanabe, Ohnuki, Narita, Ichisaka, Tomoda and Yamanaka, 2007), blood mononuclear cells (Staerk, et al., 2010), or urine cells (Zhou, et al., 2012) from a patient or a healthy individual. These iPSCs can be differentiated using defined culture

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conditions to disease-relevant cell type(s), for example, neurons from neurological disease patients. Through comparison with healthy iPSC-derived cells, patient iPSC-derived cells can be used to identify disease phenotypes at different levels, including molecular profiles, cellular features, and physiological functionality.

Before the birth of iPSC technology and iPSC-based modeling systems, animal models, primary neural cells and immortal cell lines have contributed tremendously to our understanding of neurological diseases. However, these models all have limitations that lead to desire of developing a better modeling system.

For animal models, species difference creates a barrier to fully recapitulate disease phenotypes in animal models, therefore causing high rate of failure in animal-model-based drug development. Using primary cells are often hurdled by their inaccessibility to fresh patient tissues and this is especially true for brain tissues. Postmortem tissues are more available than fresh tissues, however, neural cells are extremely sensitive to oxygen and blood supply, which makes isolation of them from postmortem tissues technically challenging. Moreover, primary cells, in particular postmitotic neural cells, such as primary neurons and oligodendrocytes, are difficult to expand to meet the need of experiments under in vitro culture environment. Although cell lines generated from tumors proliferate better than primary cells, they are distinct from non-tumor cells due to their oncogenic characteristics.

iPSC-based disease modeling has been increasingly popular for studying neurological diseases given its advantages compared with the above mentioned modeling systems. iPSCs reprogrammed from human somatic cells are originated from human, therefore avoiding the concerns of species differences as associated with using animal models. iPSCs can be expanded efficiently and provide unlimited resource for subsequent differentiation into cell types of interest. Most importantly, iPSCs reprogrammed from patient somatic cells retain their original genomic features, such as gene mutations and chromosome abnormalities. These genomic features can be maintained after differentiation, therefore can be used to study the effects of certain genomic defects on cellular functions. This is particularly advantageous for drug development. Liu et. al. demonstrated the advantage of using iPSC-based system for drug development by showing that treating iPSC-derived neurons with potential drugs for Alzheimer's disease can better reflect biomarker changes in real patients(Liu, et al., 2014). With the recent advances of genome editing technologies, especially TALEN (Boch, et al., 2009, Moscou and Bogdanove, 2009) and CRISPR/Cas9 (Cong, et al., 2013, Wiedenheft, et al., 2012), iPSCs can be manipulated from a single nucleotide change of one gene of interest to a specific fragment deletion on a disease-associated chromosome. Flexibility of genome editing in iPSCs allows us to compare molecular and cellular phenotypes at the same genetic background, leading to more relevant conclusions about disease mechanisms.

As iPSC-based disease modeling has contributed tremendously to our understanding of neurological diseases, in this review, we present an overview about developmental principles of each neural cell type, and summarize how researchers generate various types of neural cells from iPSCs using knowledge gained from developmental biology. We will review the

applications of iPSC-derived neural cell types in neurological diseases modeling. Limitations and challenges of current neural cell derivation protocols and iPSC-based disease models will also be discussed.

Neural Stem cells

Neural stem cells provide life-long resource of neurons and glial cells in the brain, and serve as the root for adult neurogenesis to develop, repair and modulate nervous system functions (Gage and Temple, 2013). Dysfunctions of neural stem cells contribute to a variety of neurological and psychiatric disorders. Now combined with iPSC technology, neural stem cells can be generated from iPSCs through mimicking in vivo developmental environment and applied to neurological disease modeling.

Neural stem cells were initially generated from iPSCs through either embryo body (EB) formation or co-culturing with stromal cells. Due to the complexity and low efficiency of these methods, new approaches were developed using small molecules that target key pathways identified in developmental biology. Li et al. found that synergistic inhibition of glycogen synthetase kinase (GSK3), transforming growth factor β (TGF β) and NOTCH pathways using small molecules CHIR99021 and SB431542 can efficiently induce human ESCs to NSCs within one week (Li, et al., 2011). These NSCs express characteristic markers, including N-Cadherin, SOX2 and NESTIN. Additionally, neural differentiation potential of these NSCs can be maintained in the presence of LIF. Several other studies also succeeded in NSC differentiation through small molecule treatment, using slightly different cocktails. Dorsomorphin and Compound E are commonly used in addition to CHIR99021 and SB431542 to induce NSC from iPSCs (Liu, et al., 2012).

Using iPSC-derived NSCs, a wide range of neurological diseases have been studied, including not only monogenic but also complex neural disorders. Using small molecules including CHIR99021, SB431542, dorsomorphin and Compound E, Liu et al. generated NSCs from Parkinson disease patient-derived iPSCs, which carried LRRK2 mutation from the patient (Liu, Qu, Suzuki, Nivet, Li, Montserrat, Yi, Xu, Ruiz, Zhang, Wagner, Kim, Ren, Li, Goebel, Kim, Soligalla, Dubova, Thompson, Yates, Esteban, Sancho-Martinez and Izpisua Belmonte, 2012). They found a novel phenotype in patient iPSCs derived NSCs, which exhibited LRRK2 mutation-correlated nuclear-architecture defects and increased proteosomal stress. However, as iPSCs derived cells were shown to possess features similar as fetal cells, the role of NSC in ageing diseases needs to be carefully evaluated in iPSCs-based modeling system. Ageing-inducing compounds, for example, Progerin, MG132, and concanamycin A, were identified to facilitate ageing of iPSCs-derived neural cells, which may benefit the modeling of ageing related neurological diseases (Cooper, et al., 2012, Miller, et al., 2013, Nguyen, et al., 2011).

iPSC-derived NSCs were also applied to delineate complex chromosomal changes in neurological diseases. Yoon et al. identified CYFIP as the mediator of 15q11.2 copy number variation associated neuropsychiatric diseases, schizophrenia and autism, which affects iPSCs derived NSC cytoskeleton organization therefore NSC deficits (Yoon, et al., 2014). Murai et al. looked into detailed mechanism of schizophrenia using iPSCs derived NSCs and

identified alteration of miR219 biogenesis due to TLX up-regulation in SCZ iPSCs derived NSCs, which leads to NSC proliferation decrease that may contribute to SCZ pathogenesis (Murai, et al., 2016). Using a similar approach, a chromosome abnormality associated with William syndrome has been delineated. In this study, Chailangkarn et al. observed defects of proliferation and survival in patient iPSCs derived NSCs (Chailangkarn, et al., 2016). Most importantly, they narrowed down the affected chromosome region to a single gene candidate, FZD9 that contributes to cellular phenotypes. Therefore, iPSC-derived NSCs have provided novel insights into neurodevelopmental and neuropsychiatric disorders.

Neurons

Neurons, as the basic working unit in the brain, are affected in the majority of neurological diseases. iPSC-derived neurons have attracted great interest for modeling neurodegenerative diseases. In order to model these diseases using iPSCs, the first challenge is to derive disease-relevant neuronal subtypes. The rich diversity of neuronal subtypes is determined by complex genetic and environmental cues. During embryogenesis, morphogenesis of neuroectoderm is specified by a combination of morphogens along two axes: rostro-dorsal axis by WNT, FGFs and retinoic acid (RA); dorso-ventral axis by WNTs, BMPs and SHH (Liu and Zhang, 2011). With this knowledge from developmental biology, researchers have used morphogens and growth factors to generate subtype- and region-specific neurons from iPSCs.

Glutamatergic neurons can be generated as default in the absence of exogenous morphogens in neuroepithelial differentiation medium, due to ventral fate repression and dorsal fate induction by endogenous WNTs in the differentiation medium (Li, et al., 2009). GABAergic neurons, as one of the major neuronal subtypes in ventral forebrain, can be induced from iPSCs through SHH activation and WNTs inhibition (Liu, et al., 2013, Xu, et al., 2010). Administration of SHH or Smoothened agonist Purmorphamine together with nerve growth factor (NGF) generates basal forebrain cholinergic neurons (BFCNs) (Duan, et al., 2014), while ventralization by SHH in combination with caudalization by RA is a general principle for motor neuron (MN) induction (Du, et al., 2015, Hu and Zhang, 2009).

These specific types of neurons developed from iPSCs have been applied to neurological disease modeling and disease phenotype characterization. Amyotrophic lateral sclerosis (ALS) is a motor neuron disease that specifically exhibit motor neuron degeneration. ALS patient iPSC-derived motor neurons were utilized to model ALS in vitro. Multiple groups have differentiated motor neurons using iPSCs derived from familial ALS patients and observed diverse phenotypes, ranging from mitochondrial dysfunctions (Kiskinis, et al., 2014) to neurite degeneration (Chen, et al., 2014). Alzheimer disease (AD) and Parkinson disease (PD) are also two hot topics for disease modeling using iPSC-derived neurons. iPSC-derived DA neurons have been used to model both sporadic and familial forms of PD, as DA neuronal death is considered the major cause for loss of movement control in PD patients. α -Synuclein, as a major gene linked to sporadic PD, has been shown to lead to iPSC-derived DA neuron defects, including decrease of dopamine level and network activity (Woodard, et al., 2014). Besides α -Synuclein, other genetic factors are also studied using iPSC-derived neurons, for example, LRRK2, PARK2, and PINK1 in familial PD, and GBA1

SCNA SNP in sporadic PD (summarized by Shi *et al.* (Shi, Inoue, Wu and Yamanaka, 2017)). Different from PD, AD patients exhibit BFCN vulnerability. AD patient iPSC-derived BFCNs have been shown to exhibit increased cell apoptosis in response to glutamate stimulation (Duan, Bhattacharyya, Belmadani, Pan, Miller and Kessler, 2014). More interestingly, this AD-associated BFCN phenotypes are related with APOE polymorphism, which is a risk factor for sporadic AD. PSEN1 and APP mutations for familial AD and SOR1 for sporadic AD are also modeled using AD patient iPSC-derived neurons (Mungenast, et al., 2016); also summarized by Shi *et al.*(Shi, Inoue, Wu and Yamanaka, 2017)). Therefore, iPSC-derived neurons provide powerful platform for uncovering the role of risk factors for neurological diseases, which remain to explore more in the future.

Although increasing studies have demonstrated the capability of iPSC-derived neurons as faithful models for neurological diseases, neuronal differentiation protocols used by different research groups differ from each other and lead to distinct phenotypes for the same disease. Therefore, it is necessary to establish standard protocols that allow comparison of findings across studies. In addition, multiplex models still need to be developed so that factors beyond cell-autonomous effects can be explored. For example, mouse models have provided strong evidence that immune response can exaggerate neural damage in response to external or internal disturbance of homeostasis (Aktas, et al., 2007). A multiple cell type co-culture system that incorporate immune-related cell types, such as microglia, astrocytes and leukocytes may help to study cell-cell interactions in the context of related diseases. Development of multiplex system may require engineering of scaffold and extracellular matrix to mirror brain structure.

Astrocytes

Astrocytes are the most abundant cell type in the brain but have been largely ignored compared to the extensive focus in neurons until recently. With the growing knowledge of astrocyte biology, its role in neurological diseases is now increasingly appreciated (Pekny, et al., 2016, Sofroniew and Vinters, 2010). Similar to neurons, astrocytes are patterned by morphogen gradients along rostro-dorsal axis and dorsal-ventral axis and exhibit heterogeneity in terms of subtypes and regionalities.

However, the protocols of astrocytes differentiation from iPSCs remain limited. The initial astrocyte differentiation protocol was published by Krensick *et al.* (Krencik and Zhang, 2011) in 2011 and since then only a few updated protocols have been developed(Jiang, et al., 2013, Shaltouki, et al., 2013, Yao, et al., 2016). Due to the fact that astrocytes are generated at much later stage of embryonic development compared to neurons, differentiation of astrocytes from iPSCs also takes longer time than neurons. Krensick et al. used different combinations of RA, FGF8, and SHH to pattern astrocytes at neural epithelial stage. Roybon *et al.* generated spinal cord astrocytes in shorter time (80 days versus 180 days by Krensick protocol) and they were able to mature astrocytes using FGF1/FGF2, which express higher levels of glutamate transporter EAAT2 as an indicator of glutamate uptake function(Roybon, et al., 2013).

Modeling neurological diseases using iPSC-derived astrocytes can be traced back to 2012. Joupéri *et al.* observed vacuolation in Huntington disease patient iPSC-derived astrocytes as well as patient peripheral lymphocytes, suggesting a novel phenotype of Huntington disease (Joupéri, et al., 2012). Astrocytes derived from ALS patient iPSCs that carry a transactive response DNA binding protein (TDP-43) mutation exhibit cell autonomous defects, including TDP-43 proteinopathies and cell death, but no adverse effects on motor neurons were observed (Serio, et al., 2013). iPSC-derived astrocytes have also been used to model AD. One group found that AD iPSC-derived astrocytes exhibit distinct morphology from normal astrocytes with less complexity and aberrant marker localization (Jones, et al., 2017). Another group took advantage of single-cell analysis and identified astrocyte as a significant contributor to A β accumulation (Liao, et al., 2016). As another dementia disease like AD, frontotemporal dementia (FTD) iPSC-derived astrocytes have been shown to exert non-cell autonomous effects on neurons, causing increased oxidative stress and transcriptional profile changes in previously healthy neurons (Hallmann, et al., 2017). Zhang *et al.* developed a 3D culture system using hydrogel as the matrix and included iPSC-derived NPCs, neurons and astrocytes to study Rett syndrome. They demonstrated the migration capability towards astrocytes are impaired in the disease iPSC-derived NPCs and neurons (Zhang, et al., 2016).

Although primary astrocyte diseases are very rare, most of them are fatal. Leukodystrophy diseases, such as Alexander disease (AxD), is caused by dominant-negative mutation of GFAP gene that is predominantly expressed in astrocytes (Quinlan, et al., 2007). This disease allows researchers to examine the role of GFAP in astrocytes beyond a cell type specific marker. Neuromyelitis optica (NMO) disease patients also exhibit severe demyelination particularly in spinal cord and optic nerve. It is an inflammatory disease caused by pathogenic auto-antibodies (NMO-IgG) against astrocyte aquaporin-4 (AQP4) (Marignier, et al., 2010). Studying primary astrocyte diseases may facilitate our understanding the role of astrocytes in the nervous system.

Current challenges of modeling neurological diseases using iPSC-derived astrocytes still remain to be addressed. As astrocytes are highly heterogeneous, development of subtype specific astrocytes would allow more accurate disease modeling. Current astrocyte differentiation protocols can be further tailored to improve astrocyte purity and differentiation efficiency. And identification of astrocyte and subtype-specific surface markers may improve homogeneity of astrocytes being derived from iPSCs.

Oligodendrocytes

Oligodendrocytes (OLs) form and maintain myelin sheath by extending their membrane to nearby axons of the neurons, which ensures efficient action potential transduction along axons. Oligodendrocyte progenitors (OPCs) arise from ventricular zone of the brain and spinal cord under induction of SHH during early development (Goldman and Kuypers, 2015, Spassky, et al., 2001). These OPCs migrate throughout the brain, mainly white matter, following guidance and substrate cues, such as chemoattractant and extracellular matrix (Milner, et al., 1997, Niehaus, et al., 1999, Tsai, et al., 2003). After arriving at the final destination, OPCs mature into OLs and myelinate axons under tight regulation by local environment, especially neuronal activities (Bradl and Lassmann, 2010).

Oligodendrocyte differentiation from iPSCs initially followed methods of ESC differentiation, including sequentially induction of neural epithelial cells by RA and FGF, ventralization using SHH, and administration of PDGF to improve OPC expansion (Hu, et al., 2009, Izrael, et al., 2007, Liu, et al., 2011, Wang, et al., 2013). More recent optimization of the protocol induces OL lineage restricted transcription factors OLIG2 and NKX2.2 within 12 days of differentiation, which greatly shortens the time frame of OL fate determination (Douvaras, et al., 2014). It also extends the period of proliferation of OPCs by using PDGF starting from Day 20 of differentiation, giving large yield of O4⁺ OPCs that can efficiently myelinate in vivo. Although much shorter than previous protocols, it still takes ~70 days to generate large amount of OPCs. Recently, Ehrlich et al. screened 7 transcriptional factors enriched in OLs and identified a combination of three, SOX10, OLIG2, NKX6.2, that was able to improve OPC differentiation to yield up to 70% O4⁺ from iPSC-derived NPCs in 28 days (Ehrlich, et al., 2017). The in vivo myelination capability was also proved in this study. Rapid and efficient generation of OPCs will benefit the understanding of the role of OPCs and OLs in neurological diseases.

High metabolic rates and toxic side product, such as hydrogen, make OLs one of the most vulnerable cells in CNS. Exposure to oxidative stress, inflammatory cytokines, or energy deficiency can induce OL death or affect OPC proliferation and apoptosis, therefore various neurological diseases are coupled with myelination defects (Singh, et al., 1998). Metabolic disturbance of oligodendrocytes can affect OL directly and cause demyelination as secondary effect. Although PSC-derived OPCs have been explored extensively for rescuing a variety of neurological diseases (Kawabata, et al., 2016, Thiruvalluvan, et al., 2016, Wang, Bates, Li, Schanz, Chandler-Militello, Levine, Maherali, Studer, Hochedlinger, Windrem and Goldman, 2013), and promising results are expected to be translated to clinical trials in the next few years, neurological disease modeling using iPSC-derived OPCs is still quite limited. Two studies succeeded in modeling the same disease using iPSC-derived oligodendrocytes, which is Pelizaeus-Merzbacher disease (PMD). PMD is an X-linked leukodystrophy disease caused by mutations of proteolipid protein 1 (PLP1) gene. Both groups identified abnormalities in iPSC-derived oligodendrocyte lineage cells. Numasawa *et al.* demonstrated ER stress associated with PMD iPSC derived oligodendrocytes (Numasawa-Kuroiwa, et al., 2014). Nevin *et al.* observed versatile cellular phenotypes depending on mutation types of the PLP gene, point mutation, duplication/triplication or deletion (Nevin, et al., 2017). The limited number of iPSC-derived oligodendrocyte models is probably due to the requirement of large number of OPC for in vitro functional experiments and the difficulty of maintaining OPCs alone in vitro. With the progress of differentiation protocols, we anticipate more disease modeling studies using iPSC-derived OPCs or OLs will be delivered to improve our understanding of OPC and OL activities in pathological conditions.

Microglia

Microglia are resident macrophages in CNS. As the only immune cells in paranchyme of the brain, microglia act as the first line of defense like macrophages in peripheral tissues and regulate homeostasis of brain in response to tissue damage and invaders (Kettenmann, et al., 2011). In addition, emerging evidence suggests that microglia also exert effects on neuronal

activities, including neuronal proliferation and differentiation and synapse formation (Paolicelli, et al., 2011). The origin of microglia is different from macrophages in peripheral tissues, as recent studies show that microglia are not derived from fetal liver or bone marrow but hematopoietic stem cells in yolk sac (Ginhoux, et al., 2010, Schulz, et al., 2012). More interestingly, expansion of microglia pool is resulted from progenitor pool within the brain rather than entry of circulating monocytes (Waisman, et al., 2015).

Unlike other neural cell types that have been generated from iPSCs using a variety of protocols, derivation of microglia from iPSCs has become the focus of great interest just in recent years. Muffat *et al.* developed microglia-like cells from both human iPSCs and ESCs through inducing myeloid progenitors from EBs, mimicking yolk sac structures (Muffat, et al., 2016). These microglia-like cells express microglia-specific transcriptional factor PU.1 and surface markers IBA1, CD45 and CD11B. Most importantly, they can demonstrate phagocytosis capability by uptaking fluorescent beads. Pandya *et al.* published a new microglia differentiation protocol using both human and murine iPSCs (Pandya, et al., 2017). This protocol also goes through myelogenesis stage to produce CD34 and CD45 double positive hematopoietic progenitor cells followed by further induction to IBA1 and CD11B microglia progenitors. To generate mature and functional microglia, this protocol adopted astrocytes to co-culture with progenitors, which give rise to microglia that express a comprehensive set of lineage-defined markers, including HLA-DR, CD45, TREM-2 and CX3CR1.

As microglia cannot be supplied by circulating monocytes, their maintenance and proliferation rely on brain-resident progenitors. The limitation of supply makes microglia vulnerable to changes of brain environment. Although there is no neural disease identified to be caused primarily by microglia malfunction so far, pathological conditions as well as ageing are almost always associated with microglia polarization. These disorders include stroke, neuropsychiatric diseases, and ageing-related diseases (Prinz and Priller, 2014). In the study by Muffat *et al.*, the authors further explored the role of microglia in disease context using their home-made protocol (Muffat, Li, Yuan, Mitalipova, Omer, Corcoran, Bakiasi, Tsai, Aubourg, Ransohoff and Jaenisch, 2016). They found Rett syndrome patient iPSCs-derived microglia exhibit smaller size than control cells. Considering the role of microglia in immune response, instead of studying microglia alone, it would be more interesting to explore the impact of microglia on neural cells in response to stimuli, such as inflammatory cytokines and oxidative stress.

Limitations of iPSCs

iPSC provides promising tools for neuroscientists to study neurological diseases, however, it is noteworthy that iPSC-based modeling system is not perfect yet. Genetic and epigenetic variations of iPSCs existing between different iPSCs lines and clones may limit the accountability of iPSC-based disease modeling.

Genetic variations, including aneuploidy, copy number variation (CNV), and single nucleotide variation (SNP) can be introduced to iPSCs during reprogramming and maintenance. These genetic variations of iPSCs may also originate from source cells due to

their heterogeneity and be passed to iPSCs. Karyotyping abnormalities occurs in around 13% of iPSCs and hESCs, with trisomy of chromosome 12 being the most common (Taapken, et al., 2011). As chromosome 12 harbors pluripotency gene NANOG, trisomy 12 cells may possess growth advantage over other cells and get positively selected during passaging (Mayshar, et al., 2010, Taapken, Nisler, Newton, Sampsel-Barron, Leonhard, McIntire and Montgomery, 2011). CNVs and SNPs also exhibit location preferences, which confers advantage of certain population during iPSC propagation and maintenance. Epigenetic variations, such as aberrant DNA methylation at specific loci, can be generated during reprogramming and maintenance. In addition, epigenetic signatures from source cells may reside in iPSCs due to incomplete reprogramming (Bar-Nur, et al., 2011, Kim, et al., 2010).

The genetic and epigenetic variations of iPSCs could cause variations in differentiation capability and phenotypical changes between iPSCs lines, and even different clones generated from the same source line. Genetic or epigenetic variations at certain important developmental loci make some iPSCs lines easier to be differentiated into target cell types than other lines (Hu, et al., 2010, Miura, et al., 2009). They can also ameliorate expected disease phenotypes or exhibition of phenotypes irrelevant to the disease.

To cope with problems caused by genetic and epigenetic variations in iPSCs and their effects on disease modeling, two strategies can be applied. First strategy is to minimize the variations between iPSC lines through using non-integrating reprogramming method, which can avoid transgene insertion into the genome, therefore maintaining genome integrity. During maintenance, it is important to monitor variations of iPSC lines, such as karyotype status of iPSC lines, gene integrity of pluripotency and cancer-related genes, and methylation status of certain hotspot DNA loci. Abnormal iPSC lines should not be used for downstream studies.

The second strategy to minimize iPSC variations is to use isogenic lines for disease modeling. As mentioned above, gene editing technologies, especially TALEN and CRISPR/Cas9 techniques, enable precise editing of target genes. Isogenic lines including creating a mutation site in a wild-type iPSC line and correction of mutation sites in a disease iPSCs line can be included for phenotype confirmation, which would greatly eliminate background noise coming from line-to-line variation and increase the accountability of iPSC-based disease modeling.

Conclusions

Modeling neurological diseases using iPSCs provide valuable insights into disease mechanisms and unique opportunity for developing therapies to treat these diseases. Reliable modeling system depends on robust cell differentiation protocols and bona fide cell types being derived. Although current techniques are able to produce all types of neural cells that exhibit basic cellular and functional characteristics, further refining the protocols is needed to derive neural cells with better defined subtypes and identities. In addition, 3D culturing system that integrates various neural cells is flourishing. Combined with genome editing tools, especially CRISPR/Cas9 technique, and 3D organoids, modeling of genetically

complex neural disorders will become prevalent through studying pathological mechanisms in a 3D, physiologically relevant context with isogenic controls.

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Fig 1. A schematic for iPSC modeling of neurological diseases

The logical path of modeling neurological diseases using patient-derived iPSCs mainly includes the following steps. First, iPSCs are generated from patients by reprogramming somatic cells using 4 Yamanaka factors, OCT4, SOX2, KLF4, and MYC. Isogenic iPSCs can be produced through gene editing technology, such as CRISPR/Cas9. iPSCs, including isogenic iPSCs, will be induced to neural cell type(s) of interest, for example, neurons. These iPSC-derived neural cells can be applied to disease-relevant phenotypes studies. Drugs can be developed to target specific phenotype identified in the previous step for disease therapy.

Fig 2. A schematic for neural cell differentiation from iPSCs

iPSCs can be induced to neural progenitor cells (NPCs) that possess multipotency to multiple neural lineages. With additional induction, NPCs can give rise to neurons, astrocytes and oligodendrocytes. Microglia can be induced from myeloid progenitors generated from iPSCs. Each neural cell type can be used to model relevant neurological diseases.