Integrative Research of Induction of Pluripotent Stem Cells

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Abstract
Since the Japanese scientist Shinya Yamanaka used a viral vector to transfer the combination of 4 factors into differentiated somatic cells and reprogrammed them to obtain similar embryonic stem cells and induced pluripotent stem cells (iPSCs), it provided one integrative method for studying many medical fields. Patient-derived iPSCs have provided an opportunity to study human diseases for which no suitable model systems are available. iPSC technology has since become a major breakthrough in the field of stem cell research. With the continuous development of iPSC technology and the continuous improvement of technical levels, excellent advances have become more and more common in the basic research and medical fields of life sciences. This article reviews the development history, clinical application, and problems and prospects of iPSC, and focuses on the application of iPSC in neurological diseases.

Introduction
In 2006, Shinya Yamanaka and coworkers used unfertilized eggs and embryonic stem (ES) cells to induce "pluripotent" cells that had the ability to develop into skin, nerve, muscle, or practically any other cell type [1, 2]. The integrative method is one useful tool for studying...
many medical and biological areas. Cells obtained from human skin, blood, or other cells can be reprogrammed into induced pluripotent stem cells (iPSCs), and then used to grow liver cells, neurons, or whatever type of cell is needed to treat a disease. Recent advances in iPSC technology and lineage-specific differentiation of pluripotent stem cells have galvanized interest in exploring their application in basic research, disease modeling, drug discovery, and regenerative medicine [3]. Similar to ES cells, iPSCs have the ability to differentiate into any mature cells, and can avoid immune rejection and ethical problems associated with ES cell research [4]. Such an approach may offer distinct advantages when generating desirable cell populations ex vivo for cell-based therapy, or when enabling tissue regeneration via in situ reprogramming of tissue-resident cells to replace lost and/or damaged cells owing to diseases or injuries [5].

**Application of iPSCs in Neurological Diseases**

Most reprogramming techniques are inefficient: only a small fraction of cells end up fully reprogrammed, and iPSC vary from one strain to another. This has made it hard to establish controls in experiments. Paquet et al. [6] worked with iPSCs prepared from patients with early-onset Alzheimer disease and frontotemporal dementia (FTD) and recognized when comparing iPSCs of a patient with those from a healthy control that the cells behaved very differently in culture, probably as a result of disparities in genetic background or gene expression, and turned to gene editing. The CRISPR-Cas9 gene-editing tool, which has gained in popularity in recent years, has enabled researchers to introduce disease-associated mutations into a sample of iPSC and then compare them with the original, unedited cell lines. Other labs demonstrated a technique for introducing specific point mutations into iPSC using CRISPR, and editing just one copy of a gene, and generated cells with precise combinations of Alzheimer-associated mutations to study the effects. Although iPSCs resemble embryonic cells, they are not always ideal for studying late-onset diseases such as dementia. Ming and colleagues have used iPSC to create brain organoids, which are 3D bits of tissue that resemble developing organs. When they exposed these to Zika virus, they found that the pathogen preferentially infected neural stem cells over newly formed neurons, leading to increased death of the neural stem cells and a decrease in the volume of a layer of neurons in the cortex, resembling microcephaly.

Other groups have used iPSC to create organoids such as mini-guts and mini-livers, and the list of disease-related discoveries using iPSCs is growing. It includes showing how a gene duplication in glaucoma causes the death of nerve cell clusters, and recapitulating genetic and cellular alterations associated with Huntington disease [7].

The study of new drugs for the treatment of amyotrophic lateral sclerosis (ALS) has been seriously hampered by the lack of motor neurons and appropriate disease models in patients with ALS. As early as 2012, Egawa et al. [8] found that iPSC-derived motor neurons from familial ALS patients not only carry the TAR DNA-binding protein-43 (TDP-43) mutation, but also can form cytoplasmic aggregates that have been observed in autopsies of ALS patients. ALS motor neurons are characterized by the mutant TDP-43 protein in a detergent-insoluble form that binds to the splice factor SNRNPB2. Expression array analysis revealed a slight increase in gene expression levels involving RNA metabolism, and the expression of genes encoding cytoskeletal proteins was reduced. They also found that a histone acetyltransferase inhibitor called anacardic acid rescued the abnormal ALS motor neuron phenotype. These findings showed that motor neurons from iPSCs derived from ALS patients may provide a useful tool for elucidating the pathogenesis of ALS disease and screening for candidate drugs. In 2015, Lenzi et al. [9] studied the cellular behavior of muteins using iPSC from ALS patients.
with mutated RNA-binding protein fused in sarcoma/translocated in liposarcoma (FUS). The study found that iPSCs have the ability to differentiate into spinal cord neurons, which provides an in vitro model for the physiological conditions of ALS pathogenesis. The results showed that iPSCs were from fibroblasts from FUSR514S and FUSR521C patients, and fibroblasts were not available in the case of severe FUSP525L mutations. Transcription activator-like effector nuclease-directed mutagenesis can trigger heterozygous and homozygous iPSC lines. It was also found that abnormal localization and recruitment of FUS stress granules (SGs) were characteristic of FUS muteins. This characteristic manifestation occurs only when undifferentiated iPSCs and spinal cord neurons induce stress. In addition, the amount of incorporated SG is proportional to the amount of cytoplasmic FUS, which is strongly related to the cytoplasmic delocalization phenotype of the different mutants. Through this study, we believe that iPSCs can be used to study the association between FUS mutations, the molecular mechanisms of SG formation and the pathogenesis of ALS.

Parkinson disease (PD) is a degenerative disease of the nervous system, and it is a common extrapyramidal disease in the elderly. Some central nervous system degenerative diseases such as progressive supranuclear palsy, striatum nigra degeneration, Shy-Drager syndrome, and olive pontine cerebellar atrophy have PD-like symptoms [10]. The goal of stem cell therapy in PD is to rebuild the local synapse formations and/or release dopamine and cytokines in the putamen [11]. However, iPSCs are expected to provide an alternative donor cell population, because they are capable of self-renewal and multipotency. According to previous reports, dopaminergic neurons have been developed from iPSC, and ES cells were tested successfully in the brains of rats and PD monkeys. Researchers from Kyoto University have developed a method for isolating dopaminergic neuron progenitor cells as a cell population donor, which allows safe and effective transplantation [12]. In 2010, Hargus et al. [13] discovered that iPSCs from PD patients can differentiate into dopamine neurons under certain conditions. Soldner et al. [14] further found that transplanting the iPSCs into their rat model can alter its motor function. Since then, scholars began to study the mechanism of death of dopamine neurons in PD patients using iPSCs, and it was found that mitochondrial dysfunction is the key to neuronal death. Research into the treatment of PD with iPSCs is still ongoing, and the process will be tough and lengthy, but our research has shown that iPSCs have brought about the dawn of a cure for PD.

Globoid cell leukodystrophy (GLD), also known as Krabbe disease, is an autosomal recessive genetic disease. The infantile type is more common, with mental retardation and epileptic seizures. Kondo and Duncan [15] showed that GLD is a central and peripheral nervous system destructive demyelinating disease. It is caused by a genetic defect in the activity of the lysosomal enzyme galactose cerebroside lipase (GALC), which is necessary to maintain myelin. Hematopoietic stem cell transplantation (HSCT), including umbilical cord stem cell transplantation, is currently the only effective treatment. Although the treatment is not a cure, before onset, HSCT significantly prolonged the life of patients with GLD. In HSCT, donor-derived macrophages are thought to indirectly supply the enzyme (called “cross-correction”) to the host myelin cells. The practical tactics for myelin repair in GLD is to combine the rapid and extensive cross-correction of GALC by HSCT with stable myelination provided by the GALC-derived myelin-forming cells. In 2016, Itoh et al. [16] used the Sendai virus vector (SeVdp) to express 4 reprogramming factors: OCT3/4, SOX2, cMYC, and KLF4. The authenticity of the established iPSC lines WT-iPSC2 and WT-iPSC4 was confirmed to be 3 germ layers by the expression and differentiation of stem cell markers. The results showed that WT-iPSC2 and WT-iPSC4 may be useful cellular resources. At present, the relevant research is still underway. However, there is no doubt that iPSCs have opened a new door to GLD treatment.
Spinocerebellar ataxia type 3 (SCA3), a neurodegenerative disease, is caused by a CAG-repeat amplification in the ATXN3 gene. Hansen et al. [17] established iPSCs from 2 SCA3 patients. No integral method is used for reprogramming dermal fibroblasts and converting the SCA3 iPSC into neurons. These neuron systems had disease-causing mutations, showed considerable levels of some neuronal markers, and had a reaction to the neurotransmitters glutamate/glycine, GABA, and acetylcholine. In addition, all cultured neurons showed synchronized spontaneous calcium oscillations during 28 days of maturation and expressed the mature neuronal markers NeuN and synapsin 1. The study generated a set of SCA3 patients with iPSCs and a powerful protocol to derive relatively advanced maturation of neurons, which may potentially be valuable for the study of SCA3 disease mechanisms. In addition, related studies have found that apoptosis plays an important role in the pathogenesis of SCA3. Moreover, inhibition of the apoptosis pathway can reduce cell death. Accordingly, researchers can speculate that the intervention of the apoptotic link may be the target of SCA3 therapy. SCA3 pathogenesis and treatment methods remain unclear. However, the field of iPSC research related to SCA3 has opened the door for the diagnosis and treatment of SCA3.

FTD and other tauopathy lesions characterized by focal neuronal degeneration and protein pathogenesis are usually combined with tau mutations. However, the mechanism of neuronal loss is not clear. In order to identify molecular events linked with tau protein disease, Silva et al. [18] investigated neurons from iPSCs derived from tau-A152T variants. They stressed the importance of identifying preclinical studies of potential in-depth phenotypic human neuronal cell models and endogenous tau toxicity regulators. Through a set of biochemical and cellular assays, A152T neurons show tau accumulation, redistribution, and reduced solubility. Tau uptake is coupled to enhanced stress-induced markers and cells susceptible to proteotoxicity, excitotoxicity, and mitochondrial stress, which is mediated by CRISPR/Cas9-mediated tau targeting or by pharmacological activation of autophagy. The results reveal a particular passageway associated with neuronal vulnerability of tau-mediated disturbances, and reveal potential early disease biomarkers and therapeutic targets such as FTD and other tauopathies. This finding will be a milestone in the clinical treatment of Tau-A152T FTD.

Application of iPSCs in Cardiovascular Diseases

Recent studies have demonstrated that iPSC-derived cardiomyocytes (iPSC-CMs) generated from patients with inherited cardiovascular disorders recapitulate key phenotypic features of disease in vitro. These cells can be maintained in culture for prolonged periods of time and used for extensive biochemical and physiological analyses. By serving as models of inherited cardiac disorders, these systems have the potential to fundamentally change the manner in which cardiovascular disease is studied and new therapies are developed [19]. Engineered heart tissue has emerged as a personalized platform for drug screening. With the advent of iPSC technology, patient-specific stem cells can be developed and expanded into an indefinite source of cells. Subsequent developments in cardiovascular biology have led to efficient differentiation of cardiomyocytes (CMs), the force-producing cells of the heart. iPSC-CMs have provided potentially limitless quantities of well-characterized, healthy, and disease-specific CMs, which in turn has enabled and driven the generation and scale-up of human physiological and disease-relevant engineered heart tissues. The combined technologies of engineered heart tissue and iPSC-CMs are being used to study diseases and to test drugs, and in the process, have advanced the field of cardiovascular tissue engineering into the field of precision medicine [20]. The generation of iPSCs has opened up a new scientific frontier in medicine. The pathophysiological cellular phenotypes of genetically heritable heart diseases,
such as arrhythmias and cardiomyopathies, have been modeled on cell culture dishes using disease-specific iPSC-CMs. These model systems can potentially provide new insights into the disease mechanisms of cardiological diseases and drug discoveries [21]. Therapeutic angiogenesis has become the most effective method of curing damaged blood vessels due to ischemic diseases such as myocardial infarction (MI). However, implanting EC cells in the infarcted heart is still challenging. Liang et al. [22] studied a method of generating epithelial cells by suppressing microRNA-495 (miR-495) iPSC and evaluating its potential in the treatment of vascular MI. Through expression spectrum and calculation analysis, they identified that the antiangiogenic miR-495 belongs to the Dlk1-Dio3 miR clusters. The function of miR-495 was missed in the experiment using iPSC lentivirus transfer of the antisense sequence. iPSC pluripotency was not influenced by genetic modification. Using induction differentiation medium, the inhibition of miR-495 upgraded the expression of the EC gene in iPSCs, and the output of EC. The new epithelial cells have outstanding properties of angiogenesis, including formation of the tube, cell migration, and proliferation. MiR-495 mediated endothelial or angiogenesis gene expression by directly targeting endothelial zinc finger 1 (VEZF1). After transplantation in immunodeficient mice MI, there is a significant increase in EC-derived new blood vessels formed in the infarcted heart, a function of preventing deterioration and attenuated infarct size expansion. EC functional integration implanted into the coronary network is also enhanced. Therefore, the study proves that miR-495 represents a new target, not only to promote epithelial cells produced from iPSCs, and can be used to enhance angiogenesis and migration of iPSC-derived EC in the ischemic heart. Zhang et al. [23] have also proposed that transcription factors involved in tyrosine-induced JAK inhibitors J11, such as BACS (bone morphogenetic protein 4, activin, CHIR99021, and SU5402), can transform murine fibroblasts into inducible myocardial progenitor cells (ieCPCs). Furthermore, under the action of BACS, ieCPCs can be continuously amplified and differentiated into CMs, smooth muscle cells, and endothelial cells. A study by Cao et al. [24] found that the same kind of cardiovascular progenitor cells can be obtained from human pluripotent stem cells (hiPSC) under the action of complex cytokines, which can be self-renewed and updated. This will be of great significance in treating vascular damage due to ischemic disease.

**Application of iPSCs in Liver Diseases**

The discovery of iPSCs unraveled a mystery in stem cell research: the identification of 4 reprogramming factors for generating pluripotent stem cells without the need for embryos. iPSCs have the potential to differentiate into desired cell types, including hepatocytes, under in vitro as well as in vivo conditions given the proper microenvironment. iPSC-derived hepatocytes could be useful as an unlimited source, which can be utilized in disease modeling, drug toxicity testing, and producing autologous cell therapies that would avoid immune rejection and enable correction of gene defects prior to cell transplantation [25]. Nong et al. [26] used ultracentrifugation and ultrafiltration to separate and concentrate exosomes from conditioned media. The hiPSC-MSC-Exo was systematically injected through the inferior vena cava in a rat model of 70% warm liver ischemia-reperfusion injury. Then, the serum levels of transaminase (aspartate amino transferase and alanine amino transferase) and the expression of inflammatory factors were measured. Histological changes indicated pathological changes in the liver and inflammatory infiltration. Apoptosis of hepatocytes in liver tissue was measured using terminal deoxynucleotidyl transferase-mediated nick end labeling staining and apoptotic markers. iPSCs were efficiently induced into iPSC-MSC with typical MSC characteristics. hiPSC-MSC-Exo has exosomal labeling (CD9, CD63, and CD81) in a diameter from 50 to 60 nm. Hepatocellular necrosis and sinus congestion were significantly inhibited by the adminis-
tration of hiPSC-MSCs-Exo, and the Suzuki score was lower. The levels of aspartate amino transferase and alanine amino transferase were significantly lower in the treatment group than in the control group. Inflammatory markers such as tumor necrosis factor-α, interleukin-6, and high-mobility group box 1 were significantly reduced after administration of hiPSC-MSCs-Exo. In addition, the levels of apoptotic markers such as caspase-3 and bax were significantly reduced in the liver tissue of the experimental group, and oxidative markers such as glutathione, glutathione peroxidase, and superoxide dismutase were significantly higher than those in the control group. These data point to the role of hiPSC-MSCs-Exo in anti-apoptotic and antioxidative stress responses. Therefore, the results suggest that hiPSC-MSC-Exo may alleviate hepatic ischemia-reperfusion injury by inhibiting the inflammatory response and oxidative stress response, and the inhibition of apoptosis.

A critical shortage of donor organs for treating end-stage organ failure highlights the urgent need for generating organs from human iPSCs [27]. Despite many reports describing functional cell differentiation, no studies have succeeded in generating a 3D vascularized organ such as the liver. A study showed the generation of vascularized and functional human liver from human iPSCs by transplantation of liver buds created in vitro (iPSC-LBs). Specified hepatic cells (immature endodermal cells destined to track the hepatic cell fate) self-organized into 3D iPSC-LBs by recapitulating organogenetic interactions between endothelial and mesenchymal cells. Immunostaining and gene-expression analyses revealed a resemblance between in vitro grown iPSC-LBs and in vivo liver buds. Human vasculatures in iPSC-LB transplants became functional by connecting to the host vessels within 48 h. The formation of functional vasculatures stimulated the maturation of iPSC-LBs into tissue resembling the adult liver. Highly metabolic iPSC-derived tissue performed liver-specific functions such as protein production and human-specific drug metabolism without recipient liver replacement. Furthermore, mesenteric transplantation of iPSC-LBs rescued the drug-induced lethal liver failure model. The study was the first to demonstrate the generation of a functional human organ from pluripotent stem cells. Although efforts must ensue to translate these techniques into treatments for patients, this proof-of-concept demonstration of organ-bud transplantation provides a promising new approach to study regenerative medicine.

Generation of functional and vascularized organs from human iPSCs will facilitate our understanding of human developmental biology and disease modeling, hopefully offering a drug-screening platform and providing novel therapies against end-stage organ failure. Here, we describe a protocol for the in vitro generation of a 3D liver bud from human iPSC cultures and the monitoring of further hepatic maturation after transplantation at various ectopic sites. iPSC-derived specified hepatic cells are dissociated and suspended with endothelial cells and mesenchymal stem cells. These mixed cells are then plated onto a presolidified matrix, and they form a 3D spherical tissue mass termed a liver bud (iPSC-LB) in 1–2 days. To facilitate additional maturation, 4-day-old iPSC-LBs are transplanted into an immunodeficient mouse. Live imaging has identified functional blood perfusion into the preformed human vascular networks. Functional analyses show the appearance of multiple hepatic functions in a chronological manner in vivo [28].

Liver disease is a leading cause of death in the Western world. However, our insight into the underlying disease mechanisms and the development of novel therapeutic agents has been hindered by the limited availability of primary tissue, intraspecies variability associated with the use of animal models, and reduced long-term viability of isolated and diseased liver cells. The emergence of human iPSCs and differentiation protocols to generate hepatocyte-like cells has opened up the possibility of addressing these issues. Here, we discuss the recent progress and potential in the production of various cell types constituting the liver and their applications to model liver diseases and test drug toxicity in vitro [29].
Application of iPSCs in the Field of Ophthalmology

The study of effective and reproducible conditions for the directional differentiation of pluripotent stem cells into specific cell types is important not only for understanding early human development but also for achieving more practical applications such as in vitro disease modeling, drug discovery, and cell therapy. The differentiation of stem cells into retinal pigment epithelium (RPE) is particularly promising as a source of cells for therapeutic replacement in age-related macular degeneration. As early as 2013, Takahashi Yadai and her team [30] produced iPSCs from skin cells of 2 men with age-related macular degeneration and carried out clinical trials using the cells to create RPE cell flakes. On September 12, 2014, the doctors implanted the first RPE cell flakes into the right eye of a 70-year-old woman, and cured her age-related macular degeneration, thus enabling her to regain her sight.

iPSCs in the Study of Drug Interactions

Drug interactions can result in difficult drug safety issues if we take into account the increasing number of individuals taking a variety of drugs and the relative complexity of the evaluation of the interaction potential. For example, drug treatment based on sofosbuvir has significantly advanced the care of patients with hepatitis C virus infections. However, some reports have indicated that interactions with amiodarone may lead to serious symptomatic bradycardia, thus limiting the effectiveness of the treatment. Millard et al. [31] assessed the ability of CMs derived from iPSCs to reinteract with sofosbuvir and amiodarone in vitro, and furthermore, evaluated the feasibility of hiPSC-CM as a drug-drug interaction model system. The electrophysiological effects of sofosbuvir alone on myocardial cells are not significant, and a dose-dependent effect of the sofosbuvir-amiodarone combination may be superior. In contrast, GS-331007, the main circulating metabolite of sofosbuvir, alone or in combination with amiodarone has no effect. Further studies have suggested that the combination of sofosbuvir and amiodarone may influence calcium transient amplitudes in cells [32]. These effects were independent of the collective mechanism of direct ion channel blockage and P-glycoprotein activity. These results demonstrate that iPSC-CMs can act as a comprehensive but extensible model system to identify and evaluate cardiac pharmacodynamic drug-drug interactions. The results of this study will have an interdisciplinary significance for the study of drug interactions. However, the development of such work still needs a lot of strong evidence to support it [33].

iPSCs have also been used with some success in drug discovery: they provide a plentiful source of patient-derived cells to screen or test experimental drugs. In 2012, for example, neural stem cells obtained from people with a nerve cell development disease were used to screen nearly 7,000 small molecules and identify a potential drug for the condition. Furthermore, in 2017 a team reported generating sensory neurons from iPSC cells obtained from people with an inherited pain disorder. The researchers showed that a sodium-blocking compound reduced the excitability of neurons and decreased pain in the patients. "It would be great to use iPSC cells to predict whether people will respond to a particular drug," said Edward Stevens, a research fellow at the Pfizer Neuroscience and Pain Research Unit in Cambridge, UK, who led the work, but there will need to be much more evidence that such a strategy works. Even after a decade of reprogramming cells, researchers still do not know in detail how the process actually occurs. For now, the field is focused on systematically verifying the identity and safety of cell lines by checking their genomes, gene expression patterns, and other parameters. One such effort, the European Bank for Induced Pluripotent Stem Cells, located in Cambridge, UK, publicly launched in March 2017 its catalogue of standardized iPSC
cells for use in disease modeling. Dr. Yamanaka of Kyoto University is also involved in banking iPSCs for future therapies, collecting varieties that would be immunologically compatible across a broad population. The greatest future challenges, he says, are not scientific. Researchers are going to need strong support from the pharmaceutical industry and governments to move forward with cell therapies; for drug discovery and disease modeling, researchers must be persistent and patient.

**Difficulties and Challenges Facing iPSCs**

Over the past decade, there have been new discoveries and breakthroughs in the study of iPSCs. At the same time, we are also facing great challenges. First, the success rate of reprogrammed cells is very low. For example, in Shinya Yamanaka’s mouse study, the proportion of somatic cell reprogrammed iPSCs was 0.01–0.1%, and this inefficiency required the researchers to control the exact time, the equilibrium point, and reprogrammed genes, in order to grasp less genetic and epigenetic changes in the initial somatic population or population [34]; Secondly, the ability of the transformation of iPSC is not exactly the same; iPSC is transformed from one breed to another, which is very difficult to grasp in specific trials, which will make clinical use more difficult. Finally, for “safety” considerations, studies have proved that iPSCs and the resulting cells have some small gene changes, although at present we have no evidence indicating that the mutations are related to tumor formation. Therefore, we need to find a balance between reprogramming efficiency and tumorigenesis.

In conclusion, even after a decade of reprogramming cells, researchers still do not know how the process actually occurs. The research field is to focus on systematically verifying cell lines’ identity and safety, by checking their genomes, gene expression patterns, or other things. Like Dr. Yamanaka in Japan, many countries are establishing iPSC banks for future therapies by collecting varieties that would be immunologically compatible across a large population. The greatest future challenges are that researchers need strong support from the pharmaceutical industry and governments to move forward with cell therapies for drug discovery and disease modelling. Researchers must be persistent and patient.

**Summary and Prospects**

Due to the above 3 questions, the integrative application of iPSCs is still challenging. Between efficiency and genome fusion, researchers need to find a balance between key points. Most of the methods that do not rely on gene fusion exhibit inefficiencies, and those that rely on foreign gene fusion are faced with the problem of insufficient reprogramming and tumorigenesis. Of course, people have tried many technologies and methods. Another major strategy is to identify the iPSC proteome. Stanford University’s Wu Task Force has made significant progress in this regard [35]. Further research and new strategies will help us find the best solution to the 5 main challenges mentioned above. An interesting experiment would be to try to integrate the advantages of these strategies into a final and effective technique for reassembling iPSCs. Another approach is to use patient-derived iPSC to determine therapeutic agents for different phenotypes. For example, iPSC lines (where P63 mutations) are present in patients with ectodermal dysplasia syndrome, showing abnormal epithelial phenotypes, and small molecule compounds can treat a portion of the disease [36]. Of course, the more important things are that iPSC research and clinical applications also require national policy support, as well as strong support from the pharmaceutical industry and governments, and actively promote cell therapy, drug discovery, and disease modeling. The perseverance and
efforts of researchers and innovation are also essential for the development of iPSCs. We believe iPSCs will eventually lead us into a new era of basic research and clinical areas of medicine.

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References

1. Takahashi K, Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–676.
2. Loh YH, Wu Q, Chew JL, Vega VB, et al: The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. Nat Genet 2006;38:431–440.
3. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, et al: Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318:1917–1920.
4. Qian X, Nguyen HN, Song MM, et al: Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. Cell 2016;165:1238–1254.
5. Cheng B, Lu SL, Fu XB: Regenerative medicine in China: main progress in different fields. Mil Med Res 2016;3:140–152.
6. Paquet D, Kwart D, Chen A, Sproul A, et al: Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9. Nature 2016;533:125–129.
7. Kaye JA, Finkbeiner S: Modeling Huntington’s disease with induced pluripotent stem cells. Mol Cell Neurosci 2013;5:6:50–64.
8. Egawa N, Kitaoka S, Tsukita K, et al: Drug screening for ALS using patient-specific induced pluripotent stem cells. Sci Transl Med 2012;4:145ra104.
9. Lenzi J, De Santis R, et al: ALS mutant FUS proteins are recruited into stress granules in induced pluripotent stem cell-derived motoneurons. Dis Model Mech 2015;8:755–766.
10. Goldmann Gross R, Siderowf A, Hurting H: Cognitive impairment in Parkinson’s disease and dementia with Lewy bodies: a spectrum of disease. Neurosignals 2008;16:24–34.
11. Freed CR, Greene PE, Breeze, RE, et al: Transplantation of embryonic dopamine neurons for severe Parkinson’s disease. N Engl J Med 2001;344:710–719.
12. Takahashi J: IPS cell therapy for Parkinson’s disease. Rinsho Ketsueki 2016;57:1080–1086.
13. Hargus G, Cooper O, Deleidi M, Levy A, Lee K, Marlow E, et al: Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. Proc Natl Acad Sci USA 2010;107:15921–15926.
14. Soldner F, Hockemeyer D, Beard C, Gao Q, et al: Parkinson’s disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 2009;136:964–977.
15. Kondo Y, Duncan ID: Myelin repair by transplantation of myelin-forming cells in globoid cell leukodystrophy. J Neurosci Res DOI: 10.1002/jnr.23909.
16. Itah M, Kawagoe S, Okano HJ, Nakagawa H: Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a healthy individual: WT-iPSC2. Stem Cell Res 2016;17:16–18.
17. Hansen SK, Stummann TC, Borland H, Hasholt LF, et al: Induced pluripotent stem cell-derived neurons for the study of spinocerebellar ataxia type 3. Stem Cell Res 2016;17:306–317.
18. Silva MC, Cheng C, Mair W, Almeida S, et al: Human iPSC-derived neuronal model of Tau-A152T frontotemporal dementia reveals Tau-mediated mechanisms of neuronal vulnerability. Stem Cell Reports 2016;7:325–340.
19. Savla JJ, Nelson BC, Perry CN, Adler ED: Induced pluripotent stem cells for the study of cardiovascular disease. J Am Coll Cardiol 2014;64:512–519.
20. Tzatzalos E, Abilez OJ, Shukla P, Wu JC: Engineered heart tissues and induced pluripotent stem cells: macro- and microstructures for disease modeling, drug screening, and translational studies. Adv Drug Deliv Rev 2016;96:234–244.
21. Tanaka A, Yuasa S, Node K, Fukuda K: Cardiovascular disease modeling using patient-specific induced pluripotent stem cells. Int J Mol Sci 2015;16:1889–18922.
22. Liang J, Huang W, Cai W, Wang L, et al: Inhibition of microRNA-495 enhances therapeutic angiogenesis of human induced pluripotent stem cells. Stem Cells 2017;35:337–350.
23. Zhang Y, Cao N, Huang Y, Spencer CI, Fu JD, et al: Expandable cardiovascular progenitor cells reprogrammed from fibroblasts. Cell Stem Cell 2016;18:368–381.
24 Cao N, et al: Highly efficient induction and long-term maintenance of multipotent cardiovascular progenitors from human pluripotent stem cells under defined conditions. Cell Res 2013;23:1119–1132.
25 Subba Rao M, Sasikala M, Nageshwar Reddy D: Thinking outside the liver: induced pluripotent stem cells for hepatic applications. World J Gastroenterol 2013;19:3385–3396.
26 Nong K, Wang W, Niu X, Hu B, et al: Hepatoprotective effect of exosomes from human-induced pluripotent stem cell-derived mesenchymal stromal cells against hepatic ischemia-reperfusion injury in rats. Cytotherapy 2016;18:1548–1559.
27 Takebe T, Sekine K, Enomura M, Koike H, Kimura M: Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 2013;499:481–484.
28 Takebe T, Zhang RR, Koike H, Kimura M, et al: Generation of a vascularized and functional human liver from an iPSC-derived organ bud transplant. Nat Protoc 2014;9:396–409.
29 Sampaziotis F, Segeritz CP, Vallier L: Potential of human induced pluripotent stem cells in studies of liver disease. Hepatology 2015;62:303–311.
30 Millard DC, Strock CJ, Carlson CB, Aoyama N, et al: Identification of drug-drug interactions in vitro: a case study evaluating the effects of sofosbuvir and amiodarone on hiPSC-derived cardiomyocytes. Toxicol Sci 2016;154:174–182.
31 Lagrutta A, Zeng H, Imredy J, Balasubramanian B, et al: Interaction between amiodarone and hepatitis-C virus nucleotide inhibitors in human induced pluripotent stem cell-derived cardiomyocytes and HEK-293 Cav1.2 over-expressing cells. Toxicol Appl Pharmacol 2016;308:66–76.
32 Cao L, McDonnell A, Nitzsche A, Alexandrou A, et al: Pharmacological reversal of a pain phenotype in iPSC-derived sensory neurons and patients with inherited erythromelalgia. Sci Transl Med 2016;8:335ra56.
33 Roy-Chowdhury N, Wang X, Guha C, Roy-Chowdhury J: Hepatocyte-like cells derived from induced pluripotent stem cells. Hepatol Int 2017;11:54–69.
34 Boland MY, Hazen JL, Nazor KL, et al: Adult mice generated from induced pluripotent stem cells. Nature 2009;461:91–94.
35 Shalom-Feuerstein R, et al: Impaired epithelial differentiation of induced pluripotent stem cells from EEC patients is rescued by APR-246/PRIMA-1MET. Proc Natl Acad Sci USA 2013;110:2152.