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Review

Recent advances in iPSC technologies involving cardiovascular and neurodegenerative disease modeling

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Abstract. Cardiovascular and neurodegenerative diseases are the most common health threats in developed countries. Limited cell derivation and cell number in cardiac tissue makes it difficult to study the cardiovascular disease using the existing cardiac cell model. Regarding the neurodegenerative disorders, the most potential sources of cell therapeutics such as fetal-derived primary neurons and human embryonic stem cells (ESCs) are associated with ethical or technical limitations. The successful derivation of human-induced pluripotent stem cells (iPSCs) by de-differentiation of somatic cells offers significant potential to overcome hurdles in the field of the replacement therapy. Human iPSCs are functionally similar to human embryonic stem cells, and can be derived autologously without the ethical challenges associated with human ESCs. The iPSCs can, in turn, be differentiated into all cell types including neurons, cardiac cells, blood and liver cells, etc. Recently, target tissues derived from human iPSCs such as cardiomyocytes (CMs) or neurons have been used for new disease modeling and regenerative medicine therapies. Diseases models could be advantageous in the development of personalized medicine of various pathological conditions. This paper reviews efforts aimed at both the practical development of iPSCs, differentiation to neural/cardiac lineages, and the further use of these iPSCs-derived cells for disease modeling, as well as drug toxicity testing.

Key words: Induced pluripotent stem cells — Cardiovascular disease — Neurodegenerative disease — Disease modeling — Differentiation to neurons and cardiomyocytes

Abbreviations: AD, Alzheimer´s disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; BMPs, bone morphogenic proteins; CMs, cardiomyocytes; CNS, central nervous system; CVD, cardiovascular disease; DA, dopaminergic neuron; EB, embryoid body; ESCs, embryonic stem cells; FAD, familial Alzheimer´s disease; FGFs, fibroblast growth factors; iPSCs, induced pluripotent stem cells; LGTS, long QT syndrome; MNs, motor neurons; PD, Parkinson´s disease; VEGFs, vascular endothelial growth factors; vSMCs, vascular smooth muscle cells; TS, Timothy syndrome.

Introduction

Despite the therapeutic advances, the treatment of patients with cardiovascular and neurodegenerative diseases still presents significant medical problem. For example, heart failure belongs to the most common causes of death in the western countries. In USA about 5.1 million individuals suffer from the heart failure, and about 280 000 die *per* year (Go et al. 2014). Medical advances at pharmacological, interventional, and surgical levels have significantly decreased the rate of mortality at the acute stage of the disease, and prolonged life expectancy. However, current treatment strategies are unable to regenerate the affected site of the heart or to provide a definitive cure (Iglesias-García et al. 2013). Recently, neurodegenerative diseases including Parkinson´s

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disease (PD), Alzheimer´s disease (AD), Huntington´s disease, and amyotrophic lateral sclerosis (ALS) constitute an increasing threat for society. In general, they are age-related, and are characterized by the progressive loss of structure or function of neurons and glial cells in the brain. According the World Alzheimer report 2014, number of people living with dementia is estimated at 44 million, proposed to almost double in 2030 and according Health communities. Also Parkinson´s disease affects 4 million people worldwide per year according Health communities (Muangpaisan et al. 2011; Norton et al. 2014).

The mechanisms of neurological disorders are not well known due to the limited accessibility of diseased tissue, as well as an inability to link both the genetic and environmental influences with changes in central nervous system (CNS) function (Deshmukh et al. 2012; Payne et al. 2015). The common features of heterogenous groups of neurodegenerative disorders include multiple pathogenesis, cellular abnormalities, unexplained phenotypic variability, and fatal outcomes (Gao et al. 2013).

Animal models have tremendously contributed to a better understanding of disease mechanism. However, there are limitations to use of animal models in terms of an accurate recapitulation of human disease (Takahashi and Yamanaka 2013). The successful derivation of human iPSCs by the de-differentiation of somatic cells provides significant potential to overcome obstacles in the field of cardiovascular and neurodegenerative diseases (Narsinh et al. 2011).

Screening of 24 factors by the Yamanaka´s group which are sufficient to revert the somatic cells to a pluripotent state demonstrated that generation of iPSCs required a combination of only four transcription factors (Oct4, Sox2, Klf4, c-myc) (Takahashi and Yamanaka 2006). In 2007, the same group succeeded in generating human iPSCs using genes encoding the same four transcription factors (Takahashi et al. 2007). The results of this research revealed that although the developmental process was thought to be irreversible, by introducing key genes into differentiated adult cells, the cells could be reset to a state in extremely early stage of

Table 1. Modeling cardiovascular and neurodegenerative diseases by iPSCs

Disease name	Associated genes in patients	Clinical features	iPSC – differentiated cell type	References
Neurodegenerative disease				
Spinal muscular atrophy	SMN1, SMN2	Movement disorder	Motor neurons	Corti et al. 2012
Amyotrophic lateral sclerosis	SOD1, FUS, TDP-43, C9ORF72	Movement disorder	Motor neurons	Faravelli et al. 2014
Alzheimer's disease	APP, PS1, PS2, APOE	Progressive dementia	Neurons	Yagi et al. 2011; Israel et al. 2012
Parkinson's disease	GBA, LRRK2, PARK2, PARK7, PINK1, SNCA, UCHL1, MAPT, SNCAIP	Movement disorder	Midbrain dopaminergic neurons	Nguyen et al. 2011; Devine et al. 2011; Badger et al. 2014
Huntington's disease	CAG triplet repeats in huntingtin (HTT)	Movement dysfunction, cognitive abnormalities, psychiatric disturbances	Neural stem cells, astrocytes, striatal neurons	Juopperi et al. 2012; Cao et al. 2014
Cardiovascular disease				
LEOPARD syndrome	PTPN11	Lentigines, electrocardiographic abnormalities, ocular hypertelorism, pulmonary valve stenosis, abnormal genitalia, growth retardation, deafness	cardiomyocytes	Carvajal-Vergara et al. 2010
Long QT syndrome		Ventricular tachyarrhythmias (syncopes, cardiac arrest, dizziness, palpitations)	cardiomyocytes	Morreti et al. 2010
LQT 1	KCNQ1			Morreti et al. 2010; Liang and Du 2014; Davis et al. 2012
LQT 2	KCNH2			
LQT 3	SCN5A			
Timothy syndrome	CACNA1C	Ventricular tachyarrhythmias, cutaneous syndactyly, intellectual disability and seizures, etc.	cardiomyocytes	Yazawa and Dolmetsch 2013

development in which they possessed pluripotency (Okano and Yamanka 2014). This allowed researchers to reprogram mature somatic cells harvested from patients and generate an unlimited supply of pluripotent stem cells. In turn, they could be differentiated into desired cell types such as neurons or cardiac cells aiming to be used for disease modeling, drug screening and cell therapy (Fig. 1) (Abeliovich and Doege 2009).

A straightforward application of iPSCs is to establish patient-specific genetic disease models *in vitro* (Table 1). These models are useful for understanding mechanism of physiology and pathology of disease, validating therapeutic targets, and drug screening/discovery (Liang and Du 2014; Payne et al. 2015).

By differentiating patient-specific iPSCs into patient-specific cardiomyocytes, it is possible generate iPSC-based "disease in a dish" models, use them to study disease mechanism and develop new therapeutics (Yoshida and Yamanaka 2010). Also, reprogramming technology allows researchers to study the development and progression of neurodegeneration, molecular pathways characteristic for specific subtypes of neurons (dopaminergic neurons in Parkinson´s disease) in human system, and may enable the discovery of the novel early diagnostics and therapies (Marchetto et al. 2011; Onder and Daley 2012). For example, iPSCs-derived neural crest stem cells could facilitate construction of tissue engineered conduits during nerve regeneration (Wang et al. 2013). The recent description of 3D cerebral organoid cultures for recapitulating human brain development and modeling the neurodevelopmental disorder microcephaly using patient iPSCs provides an exciting example of this approach (Lancaster et al. 2013).

Methods for iPSCs generation

A tractable method for establishing iPSC lines is to transfer a combination of transcription factors (Sox2, Oct4, c-myc, Klf4 and Lin28) into the somatic cell by using proper delivery vectors (Okita et al. 2008). Significant progress has been made in identifying new strategies for enhancing the reprogramming efficiency, as well as new methods for improving the clinical safety by reducing the genomic modifications required to complete the process (Iglesias-García et al. 2013). Methods involved in the transfer of genes into the target cells can be divided into: a) integrating viral vectors (viral deliv-

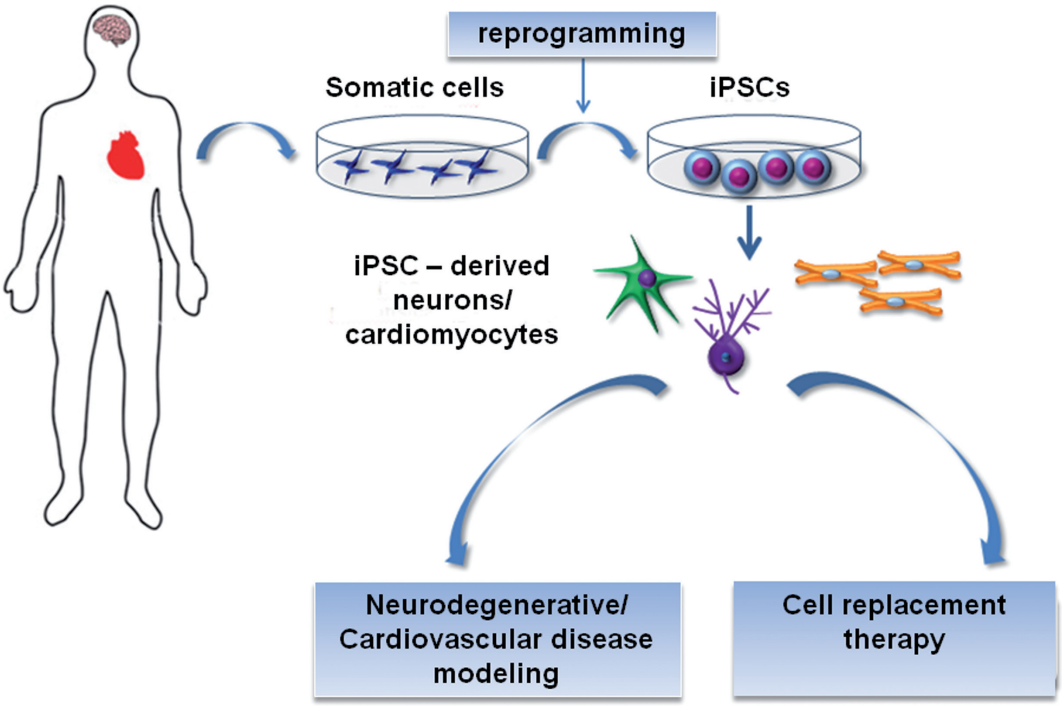

Figure 1. Generation and current applications of iPSCs. iPCSs contribute to cell replacement therapy and disease modeling. Cell replacement therapy: iPSCs can differentiate into iPSC-derived neurons (motor neurons, DA neurons, and glutaminergic neurons)/cardiomyocytes, and in combination with genome modification to repair mutated gene, direct differentiation can be a method to replace damaged cells (Abeliovich and Doege 2009). Disease modeling: patient-specific iPSCs are an abundant resource for disease modeling. Assessment of differentiated iPSC-derived cell from protein processing modification, disease-associated gene expression, and so on provides new platform for disease pathology research (Yoshida and Yamanaka 2010).

ery system, transfection of linear DNA), b) integrative free vectors (piggyBac transposon, plasmid/episomal plasmid vectors, minicircle vectors), and c) non-integrating methods (direct protein/microRNA delivery, small molecules) (Fig. 2) (Deng 2010; Tanabe et al. 2014).

Integrating methods are those in which the viral vector gets integrated into the host cell genome. The use of retroviruses and lentiviruses comes under this category. These methods have a high efficiency but possess the risks of cancer formation. Hence, with respect to that, different approaches have also been employed (Singh et al. 2015).

The most promising appear non-integrating methods. Protein transduction can completely replace the need for gene delivery. The conjugation of proteins with the short peptides responsible for cell penetration can be used for delivery of the proteins into the cells. Most mouse and human iPSCs were generated following this approach by using purified polyarginine-tagged Oct4, Sox2, Klf4, and c-myc (Kim et al. 2009). Remarkably, microRNAs alone were suggested to be capable of generating iPSCs. Transfection of mature microRNA from the miR-200c, miR-302s, and miR-369s families or infection with a lentiviral construct overexpressing the miR-302/367 clusters were reported to reprogram mouse and human adipose stromal cells or fibroblasts, respectively into iPSCs (Miyoshi et al. 2011). Small molecules (inhibitors of histon deacetylases, histone demethylases, DNA methyltransferases, etc.) can be considered as the powerful alternative for reprogramming as they can target different cellular pathways controlling cell fate, state and function. Their biological effects are typically rapid, reversible, and dose-dependent, allowing precise control over specific outcomes by fine-tuning their concentrations and combinations. Recently, Hou et al. (2013) demonstrated that chemically iPSCs could be generated from mouse somatic cells using a combination of seven small molecule compounds. The chemical reprogramming strategy appears to have potential use in generating functional suitable cell types for clinical applications.

Differentiation of cardiomyocytes and vascular smooth muscle cells from iPSCs

Efficient generation of cardiac cells represents a key goal in the therapeutic applications of iPSCs in cardiovascular disease (Iglesias-García et al. 2013). Numerous protocols, most of them based on previous embryonic stem cells (ESC) technology, have been used to differentiate iPSCs into cardiomyocytes. These protocols include: the embryoid body (EB) culture system, the monolayer culture system, and the inductive co-culture system (Dimos et al. 2008).

Cardiac differentiation of human iPSCs was firstly reported in 2009. The study outlined that both human iPSCs and ESCs have similar capacity for differentiation into nodal, atrial, and ventricular-like phenotypes. Cardiomyocytes derived from human iPSCs and ESCs share similar cardiac genes expression patterns, proliferation and sarcomeric organizations (Vidarsson et al. 2010).

In vitro differentiation of stem cells to CMs mimics the sequential stages of embryonic cardiac development. By using a series of defined growth factors guiding differentiation toward the cardiac lineage, direct differentiation protocols significantly enhancing the generation of human iPSC-derived CMs were developed. These approaches revealed guiding of CMs differentiation by sequential expression of different sets of genes in specific stages: mesoderm formation (BRY, MIXL1, FOXC1, DKK1), cardiogenic mesoderm (MESP1, ISL1, KDR), cardiac-specific progenitors (NKX2.5, GATA4, TBX5, MEF2C, HAND1/2), and CM maturation (ACTN1, MYH6, TNNT2). Three families of protein growth factors are thought to control these early stages of mesoderm formation and cardiogenesis: bone morphogenic proteins (BMPs), which are members of the transforming growth factor β superfamily; the Wingless/INT proteins (WNTs); and the fibroblast growth factors (FGFs). These factors, or their inhibitors, are expressed in the endoderm (Chow et al. 2013; Fujita and Fukuda 2014). Specifically, BMP signaling generally promotes cardiogenesis, WNTs are involved in cardiac specification, and FGFs drive mesodermal cells into myocardial differentiation.

Most commonly are cardiomyocytes generated by EBs (Nsair and MacLellan 2011). Pluripotent stem cells are cultured in suspension for about 8 days in differentiation medium, which includes EB formation. Then the EBs are further cultured on gelatin-coated dishes for another 8–10 days. The EBs contain cell types derived from mesoderm, ectoderm and endoderm. Contracting cardiomyocytes are presented within these mixed populations (Thorrez and Sampaolesi 2011). However, due to the obstacles related to both the low differentiation efficiency and purity of derived cardiomyocytes using EB system, new techniques have been developed aiming to enhance their differentiation process, namely, directing the differentiation of cardiomyocytes by using various factors on monolayer cultures. By taking advantage of a relatively uniform cellular monolayer without the complex diffusional barriers presented in EBs, application of growth factors and other interventions seems promising in the future due to the easier control and higher reproducibility. Combination of growth factors such as activing A, bone morphogenic protein 4 (BMP4), FGF 2, wingless-type mouse mammary tumor virus integration site family members 3A, and vascular endothelial growth factor (VEGF) was shown to induce cardiomyocytes differentiation with increased efficiencies (Deng 2010; Mummery et al. 2012). A more efficient way to generate cardiomyocytes is by co-culture of human ESCs/iPSCs with endodremal cells, which are mouse

embryonal carcinoma cells. This method generates cardiomyocytes with fetal ventricular characteristics in 12 days with efficiency 5–20%. More recently, small molecule-based approaches were found to be highly efficient in cardiomyocytes generation from iPSCs (Federation et al. 2014). For example, Liang and Du (2014) demonstrated an efficient and robust generation of cardiomyocytes from multiple human pluripotent stem cell lines solely *via* small molecule modulation of regulatory elements of Wnt/β-catenin signalling. Authors suggest β-catenin is essential for cardiogenesis upon pluripotent stem cells treatment with activing A and BMP4. Also, they showed the small molecules are sufficient to convert human pluripotent stem cells to cardiomyocytes efficiently when applied at the appropriate developmental stages (Narsinh et al. 2011).

Vascular cells can also participate in heart regeneration along with cardiomyocytes. By using differentiation protocols established for ESCs, recent studies have demonstrated the capacity of iPSCs to differentiate into vascular smooth muscle cells, contributing to heart repair by forming new blood vessels (Iglesias-Garcia et al. 2013). Collagen IV, retinoic acid, and the growth factors PDGF-BB and TGF-β1 have been implicated in the inducement of vascular smooth muscle cells (vSMCs) differentiation. vSMCs were previously derived from the human iPCSs from skin fibroblasts and human aortic smooth muscle cells (Wanjare et al. 2013). Wang et al. (2014) established a facile procedure to generate iPSCs from human aortic fibroblasts which were differentiated into functional vSMCs. The human iPSCs were cultured in suspension to form EBs, then the EBs were placed on Matrigel in complete SmGM2 growth medium containing a growth factor cocktail of bFGF, EGF and insulin. To further obtain functional mature vSMCs, these cells were then placed on gelatin-coated dishes in smooth muscle cell differentiation medium. At the end of the fifth day of culture differentiation, the cells elongated into spindle-shaped morphology. The contractile potential of vSMCs was tested under muscarinic agonist (carbachol) treatment. More than 50% of total tested vSMCs contracted in response to carbachol treatment.

Establishment of an excellent purification system for iPSC-derived cardiovascular cells is necessary for clinical application. Fluoresce-activated cell sorting is the most prominent method reported for selecting specific cell types, although it requires antibodies, a long processing time, and can process only small amounts of cells at one time. However, as the human heart is a large organ, current protocols still need further optimization to control *in vitro* maturation as well towards the desired subtype (Nelson et al. 2010).

Cardiovascular disease modeling

In addition to their regenerative capacity, iPSCs represent an important tool for modeling cardiovascular disease (CVD), thus allowing to study the molecular mechanisms involved in cardiac syndromes and to test specific drug targets. The advantage of using iPSCs is that it would enable modeling disease as closely to the patient's physiology and genetic as

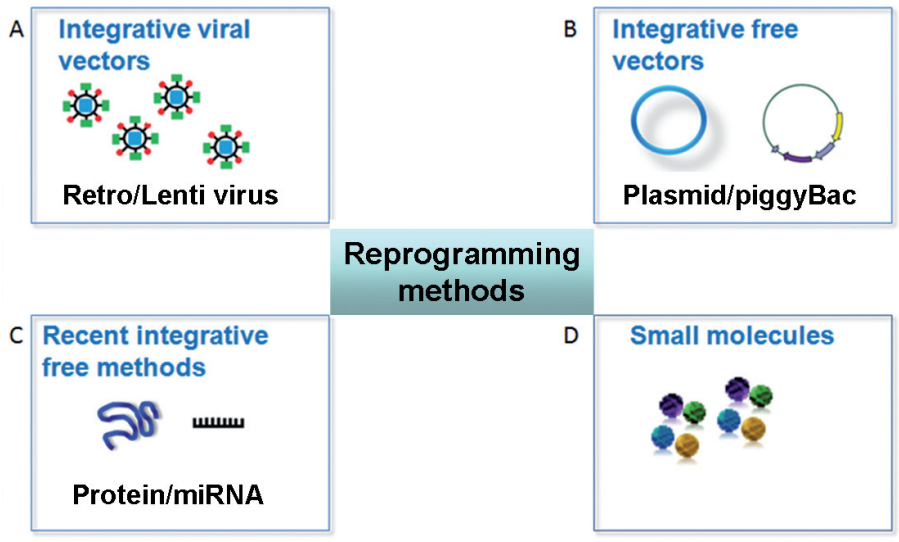

Figure 2. Methods for iPSCs generation. **A.** Retroviruses and lentiviruses are efficient gene vectors widely used in a broad range of dividing cell types (Wong and Chiu 2011). However, these vectors remain controversial due to the proviral integration, thereby increasing risk of malignant cancer transformation (Seifinejad et al. 2010; Ben-David and Benvenisti 2011). **B.** Development of integrative free vectors (plasmid, episomal plasmid, piggyBack system, minicircle vectors) has reduced the risk of genomic integration, although they suffer from low efficiency (Stadfeld et al. 2008; Woltjen et al. 2009). **C.** More recently, new methods have been developed involving protein delivery or transfection of synthetic modified miRNA representing significant advance in generating iPSCs with higher efficiency (Kim et al. 2009; Yu et al. 2009). **D.** A simple and convenient approach to manipulate epigenetic status is the use of small molecules for interferention. Thus, small epigenetic molecules retain great potential with respect to improving reprogramming and replacing exogenous reprogramming factors (Hou et al. 2013; Lu et al. 2013; Federation et al. 2014).

possible to isolating patient-specific fibroblasts (Cho et al. 2014), unlike human ESC-cardiomyocytes based disease models which can only be created from known mutations introduced into the cells. Several cardiac disease models have been established with human iPSC-cardiomyocytes demonstrating the ability to recapitulate the cellular pathogenic hallmarks of the diseases. These models are useful for understanding mechanism of physiology and pathology of disease, validating therapeutic targets, and drug screening or discovery (Liang and Du 2014).

To date, iPSC models have been used to model a large number of genetic arrhythmias including long-QT syndromes, catecholaminergic polymorphic ventricular tachycardia, arrhythmogenic right ventricular cardiomyopathy, Overlap syndrome, LEOPARD syndrome, Timothy syndrome, etc. (Chow et al. 2013). While there is a wide range of cardiovascular diseases, we decided to focus on several with well defined clinical presentation, strong genetic component, and significant research progress.

LEOPARD syndrome

Research group created the first human iPSCs model of a CVD using skin fibroblasts from a patient suffering from LEOPARD (lentigines, electrocardiogram conduction abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retardation of growth, and sensorineural deafness) syndrome, an autosomal dominant disorder caused in 90% of cases by a mutation in the *PTPN11* gene encoding the protein-tyrosine phosphatase Shp2 resulting also in myocardial hypertrophy. The generated human iPSCs-CMs exhibited increased cell size and sarcomeric organization, suggestive of the cardiac hypertrophic response, as well as aberrant RAS-MAPK signaling. When cardiomyocytes generated from the diseased iPSCs were compared with cardiomyocytes derived from human ESCs or with non-diseased iPSCs generated from a healthy brother, a significant enlargement in cell surface area, a higher degree of sarcomeric organization, and nuclear translocation of the NFATC4 transcription factor was observed, all of which correlate with the hypertrophic phenotype observed in patients (Chow et al. 2014). One limitation of the model is the cardiac differentiation from human iPSCs was performed by standard EB culture, resulting in a heterogeneous population of cells (Carvajal-Vergara et al. 2010).

Long QT syndrome

The other reported disease-specific iPSC line mimics congenital long QT syndrome (LQTS). LQTS is characterized by prolonged cardiac repolarization resulting in fatal ventricular arrhythmias. More than a dozen different types of inherited LQTS have been described, and human iPSCs have been used to study LQTS types 1, 2, 3, and 8 (Egashira et al. 2011). In long QT syndrome type 1 (LQT-1), mutations occur in the *KCNQ1* gene encoding the repolarizing potassium channel mediating the delayed rectifier I_{Ks} current. Morreti et al. (2010) provided an early example, taking skin biopsies from patients with LQT-1, reprogramming their cells into iPSCs, and then differentiating those iPSCs into cardiac cells. These patient-specific CMs recapitulated the clinical presentation of Long-QT phenotype (increased susceptibility to catecholamine-induced tachyarrhythmia, phenotype attenuated by beta-blockade). By using a similar approach, they successfully modeled long QT syndrome type 2. These studies clearly establish iPSC-derived cardiomyocytes as a powerful tool for drug discovery and personalized medicine (Liang and Du 2014).

Timothy syndrome

Yazawa and Dolmetsch (2013) derived human iPSCs from patient with Timothy syndrome (TS), a disorder in which patients suffer from long QT syndrome, autism, immune deficiency, and syndactyly due to a mutation in the *CACNA1C* gene encoding $Ca_v1.2$ L-type channel. Electrophysiological recording and calcium Ca^{2+} imaging studies of these cells revealed irregular contraction, excess Ca^{2+} influx, prolonged action potentials, irregular electrical activity and abnormal calcium transients in ventricular-like cells. In later study, the same group tested candidate drugs in TS cardiomyocytes and revealing roscovitine could successfully rescue these cellular phenotypes.

These studies indicate a tremendous potential for our increased interest in understanding of pathogenesis. However, significant hurdles still exist in modeling the more complex CVD using iPSCs technology: there are difficulties in ensuring a purified cardiomyocytes population from iPSCs through standard cardiomyocytes differentiation protocols, the complexities of reproducing a heterogeneous disease phenotype which may involve other systematic factors *in vitro* using only cardiomyocytes, and limitations of modeling essentially adult-onset diseases using iPSC-cardiomyocytes with a predominantly fetal-like phenotype (Oh et al. 2012). Resolving these obstacles will also have great impact on facilitating *in vivo* studies, widespread applications in drug discovery and development (Josowitz et al. 2011).

Differentiation of neural cells from iPSCs

In vitro differentiation and neural subtype determination of the pluripotent cells prior to transplantation, as well as the selection of cells with desire phenotype, are important parameters of every regenerative therapy (Salewski et al. 2010). Nearly all neural-related differentiation protocols

employing human ES cells have been successfully applied to iPSCs. This provides further evidence for the bioequivalence of both types of pluripotent stem cells (Hu et al. 2010; Brändl et al. 2015). The human iPSCs derived from various tissues (including skin, keratinocytes, blood cells, renal cells) have been differentiated into a variety of cells characteristic of specific neuronal subtypes so far, including motor neurons (Dimos et al. 2008), dopaminergic neurons (Sundberg et al. 2013), cholinergic, striatal and cortical GABAergic neurons, glutaminergic neurons, astrocytes, oligodendrocytes and Schwann cells (Lee et al. 2010; Israel et al. 2012; Nicholas et al. 2013; Wang et al. 2013). There are several protocols established for the generation of motor neurons (MNs) and motor neuron progenitors. The current methods for generating MNs involve EBs formation in serum-free media followed by the subsequent neural rosette formation in the presence of retinoic acid and Sonic Hedgehog, whose role is to caudalize and ventralize MN progenitors, respectively. Then, neurotrophic factors are then added to medium for the further maturation, as well as for the cells to survive. However, these methods are inefficient and require up to 60 days for developing MNs with electrophysiological properties. Other protocol describes the exogenous expression of MN-specific factors (neurogenin 2, islet-1 and LIM/homebox protein 3) in human iPSCs derived from human fibroblasts resulting into development of MNs (Karumbayaram et al. 2009; Hester et al. 2011).

Currently available methods based on differentiation of EBs or direct differentiation of adherent culture systems, are either expensive or not scalable. Badja et al. (2014) published a feeder-free method relying on the use of a chemically-defined medium that overcomes the need for EB formation and neuronal rosette isolation for neuronal precursors and terminally differentiated neuron production. This specific and efficient single-step strategy in a chemically defined medium allows the production of mature neurons in 20–40 days with multiple applications. Recently, D´Aiuto et al. (2015) developed a protocol for large-scale generation of neuronal stem cells/early neuronal progenitor cells and their differentiation into neurons. This protocol allows robust and cost-effective generation of cells above mentioned. Cells were cultured in neurobasal medium supplemented with B27 and BDNF and subsequently differentiated predominantly into vesicular glutamate transporter 1 positive neurons. iPSC-derived neurons expressed ligand-gated channels, which were functional. This protocol paves the way for automated high-throughput screening of drugs for neurological and neurodegenerative diseases.

Neurodegenerative disease modeling

Modeling a neurological disease requires developing methods aiming to mimic development for producing defined cultures of neurons or glia. In past decades, much progress was made in treating and modeling neurodegenerative diseases. However, undefined pathogenesis with multiple genetic factors and no suitable therapies for most cases have hampered further developments (Gao et al. 2013; Imaizumi and Okano 2014). Hence, it is rather challenging to study pathogenesis of human neurological disease due to the complexity of neuronal system, as well as the difficulty of culturing neurons *in vitro* (Lu and Zhao 2013). Knowledge of the onset, duration, and severity of disease at the time of tissue collection might lead to reliable correlation between the existing and the modeled disease phenotypes (Mattis and Svendsen 2011). iPSCs and their derivates may further create opportunities to identify and screen promising therapeutic compounds, and speed up the process by which drugs come through (Arbab et al. 2014).

Alzheimer´s disease

Alzheimer´s disease is one of the most common neurodegenerative disorders of the elderly, characterized by progressive memory disorientation and cognitive disturbance. As our population ages, the incidence of this disease is expected to grow dramatically, it is expected that by 2050 as many as 115 million people worldwide will be suffering from dementia (Chen and Blurton-Jones 2012). Animal model studies based on the analysis of transgenic mice overexpressing rare familiar AD-associated mutant genes is informative about the mechanisms of familiar disease, but they have not proven to be predictive for drug development (Doege and Abeliovich 2014). The pathological profile of AD is neuronal loss in the cerebral cortex and some subcortical regions (hippocampus) accompanied by massive accumulation of two types of amyloid fibril seeding senile plaques and hyperphosphorylated tau-forming paired helical filaments. The amyloid fibril is mainly composed of β-amyloid (Aβ) peptides, the 40 and 42 amino acid forms (Aβ-40 and Aβ-42) (Marchetto et al. 2011; Yagi et al. 2011). Aβ results from proteolysis of the amyloid precursor protein (APP) cleavage by β- and γ- secretase. Experiments performed on cell cultures and mice suggest that the highly toxic Aβ-42 may be overproduced in AD. The "amyloid hypothesis", stating Aβ is the cause of the disease, was difficult to verify in living nerve cells of patients (Gao et al. 2013).

The first conformation of increased Aβ-42 production of neural cells of AD patients was reported by Yagi et al. (2011). They produced iPSCs from skin fibroblasts of familial AD (FAD) patients (preselin-1 or -2 mutations) and succeeded in inducing neuronal cells. These patient-derived neuronal cells produced twice the normal level of the highly toxic Aβ-42. Following treatment of AD iPSC-derived neuronal cells with a γ-secretase modulator revealed the production of Aβ-42 was inhibited, thus this report also gave an example of the

potential use of AD iPSCs for testing drug efficacy (Doege and Abeliovich 2014).

Study by Israel et al. (2012) was focused on application of iPSCs for probing familiar and sporadic AD. Fibroblasts were derived from two patients of familiar AD caused by a duplication of the APP gene (APPDp), two patients of sporadic AD (sAD1 and sAD2), and two healthy control individuals and were reprogrammed into iPSCs. Molecular analysis of iPSCs-derived neurons indicated that APPDp and sAD2 cells had higher levels of Aβ-40, phosphor-tau, and active glycogen synthase kinase-3β (aGSK-3β), which are crucial markers for the pathological process of APP proteolysis and the formation of neurofibrillary tangles. Specific drugs were added to isolate affected neurons from APPDp and sAD2, revealing this the cells treated by β-secretase but not γ- secretase inhibitors had decreased levels of phosphor-tau and aGSK-3β. One interpretation of these findings is that β-secretase products other than Aβ may underlie tau protein pathology in the context of APP duplication. These advances achieved by iPSCs technology thus provide a promising method to study the mechanism of sporadic AD in a large group of patients (Chen and Blurton-Jones 2012).

Parkinson´s disease

Parkinson´s disease is the second most common neurodegenerative disease after AD (Okano and Yamanka 2014). Loss of dopaminergic neurons (DA) in substancia nigra of the basal ganglia is characteristic neuropathological feature. Prominent clinical features are motor symptoms (bradykinesia, tremor, rigidity, and postureal instability) and non-motor symptoms (olfactory deficits, autonomic dysfunction, depression, and sleep disorders) (Cai et al. 2014). Although, etiology of PD still remains unclear, it is believed the interactions between environmental and genetic factors are the cause of the loss of nigral DA neurons and ensuing locomotor system (Pu et al. 2012). Idiopathic PD accounts for the vast majority of parkinsonism. However, 2% to 3% of PD cases are linked to monogenic mutations. Neuronal loss is typically progressive and accompanied by α-synuclein (SNCA)-containing intraneuronal inclusions known as Lewy bodies and Lewy neuritis (Barker and Drouin-Ouellet 2014).

Currently, there is no effective medication to treat PD. Drug therapies provide only relief of symptoms and have unpredictable side effects. Although motor symptoms can be treated relatively well with L-3,4-dihydroxyphenylalanine (L-DOPA), DA agonist, enzyme inhibitors, and deep brain stimulation, effective therapies for nonmotor symptoms, such as dementia, are lacking, and disease progression cannot be counteracted. Cell therapy could be a suitable approach to treat PD (Lidvall and Kokala 2010).

Many mouse models and postmorten tissue studies have provided insight into pathogenesis of PD. However, the former consistently fail to recapitulate the cardinal features of PD and the latter are end-stage representations (Qui et al. 2013). In 2009, Soldner et al. (2009) derived human iPSCs from skin biopsies obtained from patients with idiopathic PD and developed a robust reprogramming protocol allowing the reproducible generation of patient specific stem cells with efficient removal of transgene sequences.

Using a single iPSC line as an universal control to study distinct PD-lined mutation may allow better understanding of the mechanism by which mutation affects cells and ultimately patients (Brändl et al. 2015). The first biologically relevant cellular phenotype from iPSCs derived neurons from PD patients was described by Nguyen et al. (2011). In this study, iPSCs were derived from 60-year-old female PD patient carrying a point mutation in LRRK2, the most common PD-related mutation. In the study they found that dopaminergic neurons from PD patient expressed increased levels of α-synuclein, the protein whose dysfunction unites all PD cases, and showed increased sensitivity to cellular stressors including hydrogen peroxide, MG-132 and 6-hydroxydopamine. Study of Devine et al. (2011) revealed that neurons differentiated form PD-specific iPSCs produce twice as much α-synuclein as neurons from normal iPSCs, indicating that one cause for neurodegeneration is an upregulation of α-synuclein levels. Rienhardt et al. (2011) demonstrated that neurons differentiated from iPSCs derived from patients with PD harbouring LRRK2 (G2019S) mutation exhibit multiple phenotypes including reduced axon outgrowth and increased sensitivity to stress. These PD-associated phenotypes were rescued by genetic correction (zing finger nuclease technology) of the LRRK2 mutation in the patient derived iPSCs.

Wernig et al. (2008) reported extensive differentiation of iPSC-derived neural precursor cells into glia and neurons upon their transplantation into the fetal mouse brain. Functional recovery was observed after transplantation of iPSC-derived midbrain dopamine neurons into the adult brain of Parkinsonian rats. Although iPSCs technology for PD treatment has been dramatically improved, further studies, with larger cohorts, will be necessary to confirm the phenotype observed and characterize downstream, potentially therapeutic, molecular targets (Marchetto et al. 2011).

Conclusion

iPSC-based disease models are providing valuable insight into the pathogenesis of human neurological and cardiovascular disease and cellular targets of therapeutic intervention. The ultimate goal of iPS-based disease modeling is to generate personalized iPSCs so the patient-specific cell

model can be used to validate a drug treatment strategy for patient (Cai et al. 2014). iPSCs offer many advantages over the traditional methods, which include preclinical studies mostly based on cell lines and animal models (Young et al. 2012). Nevertheless, there are a number of pressing issues needed to be addressed before iPSC technology can be extensively used for clinically relevant disease modeling (Marchetto et al. 2011). Among these questions are variability in iPSCs generation methods, variability between individuals, epigenetic/genetic instability and the ability to obtain disease-relevant subtypes of cardiomyocytes or neurons. Nowadays, there are few but important applications of iPSCs. If this field keeps on growing at the present pace, it would not take long time to expand the applications iPSCs to more biological fields to aid research and treatments (Singh et al. 2015).

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References

Abeliovich A., Doege C. A. (2009): Reprogramming therapeutics: iPS cell prospects for neurodegenerative disease. Neuron **61,** 337–339
http://dx.doi.org/10.1016/j.neuron.2009.01.024

Arbab M., Baars S., Geijsen N. (2014): Modeling motor neuron disease: the matter of the time. Trends Neurosci. **37,** 642–652
http://dx.doi.org/10.1016/j.tins.2014.07.008

Badger J. L., Cordero-Llana O., Hartfield E. M., Wade-Martins R. (2014): Parinson´s disease in a dish – Using stem cells as a molecular tool. Neuropharmacology **76,** 88–96
http://dx.doi.org/10.1016/j.neuropharm.2013.08.035

Badja C., Maleeva G., El-Yazidi C., Barruet E., Lasserre M., Tropel P., Binetruy B., Bregestovski P., Magdinier F. (2014): Efficient and cost-effective generation of mature neurons from human induced pluripotent stem cells. Stem Cells Transl. Med. **3,** 1467–1472
http://dx.doi.org/10.5966/sctm.2014-0024

Barker A., Drouin-Ouellet J. (2014): Stem cell therapies for Parkinson's disease: are trials just around the corner? Regen. Med. **9,** 553–555
http://dx.doi.org/10.2217/rme.14.43

Ben-David U., Benvenisty N. (2011): The tumorgenicyty of human embryonic and induced pluripotent stem cells. Nature Rev. **11,** 268–277
http://dx.doi.org/10.1038/nrc3034

Brändl B., Schneider S. A., Loring J. F., Hardy J., Gribbon P., Müller F. J. (2015): Stem cell reprogramming: basic implications and future perspective for movement disorders. Mov. Disord. **30,** 301–312
http://dx.doi.org/10.1002/mds.26113

Cai S., Chan Y. S., Shum D. K. (2014): Induced pluripotent stem cells and neurological disease models. Acta Physiol. Sinica **66,** 55–66

Cao L., Tan L., Jiang T., Zhu X. C., Yu J. T. (2014): Induced pluripotent stem cells for disease modeling and drug discovery in neurodegenerative diseases. Mol. Neurobiol. **52,** 244–255
http://dx.doi.org/10.1007/s12035-014-8867-6

Carvajal-Vergara X., Sevilla A., D'Souza S. L., Ang Y. S., Schaniel C., Lee D. F., Yang L., Kaplan A. D., Adler E. D., Rozov R., et al. (2010): Patient-specific induced pluripotent stem-cell derived models of LEOPARD syndrome. Nature **465,** 808–812
http://dx.doi.org/10.1038/nature09005

Corti S., Nizzardo M., Simone C., Falcone C., Nardini M., Ronchi D., Donadoni C., Salani S., Riboldi G., Magri F., et al. (2012): Genetic correction of human induced pluripotent stem cells from patients with spinal musculat atrophy. Sci. Transl. Med. **4,** 165ra162
http://dx.doi.org/10.1126/scitranslmed.3004108

D'Aiuto L., Zhi Y., Das D. K., Wilcox M. R., Johnson J. W., McClain L., MacDonald M. L., Di Maio R., Schurdak M. E., Piazza P., et al. (2015): Large-scale generation of human iPSC-derived neural stem cells/early neural progenitor cells and their neuronal differentiation. Organogenesis **10,** 365–377
http://dx.doi.org/10.1080/15476278.2015.1011921

Davis R. P., Casini S., van den Berg C. W., Hoekstra M., Remme C. A., Dambrot C., Salvatori D., Oostwaard D. W., Wilde A. A., Bezzina C. R., et al. (2012): Cardiomyocytes derived from pluripotent stem cells recapitulate electrophysiological characteristics of an overlap syndrome of cardiac sodium channel disease. Circulation **125,** 3079–3091
http://dx.doi.org/10.1161/CIRCULATIONAHA.111.066092

Deng W. (2010): Induced pluripotent stem cells: path to new medicine. EMBRO Rep. **11,** 161–165
http://dx.doi.org/10.1038/embor.2010.15

Deshmukh R. S., Kovács K. A., Dinnyés A. (2012): Drug discovery models and toxicity testing using embryonic and induced pluripotent stem-cell-derived cardiac and neuronal cells. Stem Cells Int. **2012,** 379569
http://dx.doi.org/10.1155/2012/379569

Devine M. J., Ryten M., Vodicka P., Thomson A. J., Burdon T., Houlden H., Cavaleri F., Nagano M., Drummond N. J., Taanman J. W., et al. (2011): Parkinson's disease induced pluripotent stem cells with triplication of the α-synuclein locus. Nat. Commun. **2,** 440
http://dx.doi.org/10.1038/ncomms1453

Dimos J. T., Rodolfa K. T., Niakan K. K., Weisenthal L. M., Mitsumoto H., Chung W., Croft G. F., Saphier G., Leibel R., Goland R., et al. (2008): Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science **321,** 1218–1221
http://dx.doi.org/10.1126/science.1158799

Doege C. A., Abeliovich A. (2014): Dementia in a dish. Biol. Psychiatry **75,** 558–564
http://dx.doi.org/10.1016/j.biopsych.2014.01.007

Egashira T., Yuasa Sh., Fukuda K. (2011): Induced pluripotent stem cells in cardiovascular medicine. Stem Cell Int. **2011,** 348960
http://dx.doi.org/10.4061/2011/348960

Faravelli I., Riboldi G., Nizzardo M., Simone C., Zanetta C., Bresolin N., Comi G. P., Corti S. (2014): Stem cell transplantation for amyotrophic lateral sclerosis: therapeutic potential and

perspectives on clinical translation. Cell Mol. Life Sci. **71,** 3257–3268
http://dx.doi.org/10.1007/s00018-014-1613-4

Federation A. J, Bradner J. E, Meissner A. (2014): The use of small molecules in somatic-cell reprogramming. Trends Cell Biol. **24,**179–187
http://dx.doi.org/10.1016/j.tcb.2013.09.011

Fujita J., Fukuda K. (2014): Future prospects for regenerated heart using induced pluripotent stem cells. J. Pharmacol. Sci. **125,** 1–5
http://dx.doi.org/10.1254/jphs.14R01CP

Gao A., Peng Y., Dneg Y., Qing H. (2013): Potential therapeutic applications of differentiated induced pluripotent stem cells (iPSCS) in the treatment of neurodegenerative diseases. Neuroscience **228,** 47–59
http://dx.doi.org/10.1016/j.neuroscience.2012.09.076

Go A. S., Mozaffarian D., Roger V. L., Benjamin E. J., Berry J. D., Blaha M. J., Dai S., Ford E. S., Fox C. S., Franco S., et al. (2014): Heart disease and stroke statistics--2014 update: a report from the American Heart Association. Circulation **129,** e28–92
http://dx.doi.org/10.1161/01.cir.0000441139.02102.80

Hou P., Li Y., Zhang X., Liu C. H., Guan J., Li H., Zhao T., Ye J., Yang W., Liu K., et al. (2013): Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science **341,** 651–654
http://dx.doi.org/10.1126/science.1239278

Hester E. M., Murtha M. J., Song S., Rao M., Miranda C. J., Meyer K., Tian J., Boulting G., Schaffer D. V., Zhu M. X., et al. (2011): Rapid and efficient generation of functional motor neurons from human pluripotent stem cells using gene delivered transcription factor codes. Mol. Ther. **19,** 1095–1912
http://dx.doi.org/10.1038/mt.2011.135

Hu B. Y., Weick J. P., Yu J., Ma L. X., Zhang X. Q., Thomson J. A., Zhang S. C. (2010): Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. Proc. Natl. Acad. Sci. USA **107,** 4335–4340
http://dx.doi.org/10.1073/pnas.0910012107

Chen W. W., Blurton-Jones M. (2012): Concise review: Can stem cells be used to treat or model Alzheimer disease? Stem Cells **30,** 2612–2618
http://dx.doi.org/10.1002/stem.1240

Cho G. S., Fernandez L., Kwon C. (2014): Regenerative medicine for the heart: perspectives on stem-cell therapy. Antioxid. Redox Signal. **21,** 2018–2031
http://dx.doi.org/10.1089/ars.2014.6063

Chow M. Z., Boheler K. R., Li R. A. (2013): Human pluripotent stem cell-derived cardiomyocytes for heart regeneration, drug discovery and disease modeling: from the genetic, epigenetic, and tissue modeling perspectives. Stem Cell Res. Ther. **4,** 97
http://dx.doi.org/10.1186/scrt308

Iglesias-García O., Pelacho B., Prósper F. (2013): Induced pluripotent stem cells as a new strategy for cardiac regeneration and disease modeling. J. Mol. Cell Cardiol. **62,** 43–50
http://dx.doi.org/10.1016/j.yjmcc.2013.04.022

Imaizumi Y., Okano H. (2014): Modeling human neurological disorders with induced pluripotent stem cells. J. Neurochem. **129,** 388–399
http://dx.doi.org/10.1111/jnc.12625

Israel M. S., Yuan S. H., Bardy C., Reyna S. M., Mu Y., Herrera C., Van Gorp S., Nazor K. L., Boscolo F. S., Carson C. T., et al. (2012): Probing sporadic and familiar Alzheimer's disease using induced pluripotent stem cells. Nature **482,** 216–220

Josowitz R., Carvajal-Vergara X., Lemischka I. R., Gelb B. D. (2011): Induced pluripotent stem cell-derived cardiomyocytes as models for genetic cardiovascular disorders. Curr. Opin. Cardiol. **26,** 223–229
http://dx.doi.org/10.1097/HCO.0b013e32834598ad

Juopperi T. A., Kim W. R., Chiang CH., Yu H., Margolis R. L., Ross C. A., Ming G. L., Song H. (2012): Astrocytes generated from patient induced pluripotent stem cells recapitulate features of Huntington's disease patient cells. Mol. Brain **5,** 17
http://dx.doi.org/10.1186/1756-6606-5-17

Karumbayaram S., Novitch B. G., Patterson M., Umbach J. A., Richter L., Lindgren A., Conway A. E., Clark A. T., Goldman S. A., Plath K., et al. (2009): Directed differentiation of human-induced pluripotent stem cells generates active motor neurons. Stem Cells **27,** 806–811
http://dx.doi.org/10.1002/stem.31

Kim D., Kim C., Moon J., Chung Y., Chang M., Han B. S., Ko S., Yang E., Cha K. Y., Lanza R., Kim K. S. (2009): Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell **4,** 472–476
http://dx.doi.org/10.1016/j.stem.2009.05.005

Lancaster M. A., Renner M., Martin C. A., Wenzel D., Bicknell L. S., Hurles M., Homfray T., Penninger J. M., Jackson A. P., Knoblich J. A. (2013): Cerebral organoids model human brain development and microcephaly. Nature **501,** 373–379
http://dx.doi.org/10.1038/nature12517

Lee G., Chambers S. M., Tomishima M. J., Studer L. (2010): Derivation of neural crest cells from human pluripotent stem cells. Nature Protocols **5,** 688–701
http://dx.doi.org/10.1038/nprot.2010.35

Liang P., Du J. (2014): Human induced pluripotent stem cell for modeling cardiovascular diseases. Regen. Med. Res. **2,** 4
http://dx.doi.org/10.1186/2050-490X-2-4

Lidvall O., Kokala Z. (2010): Stem cells in human neurodegenerative disorders – time for clinical translation? J. Clin. Invest. **120,** 29–40
http://dx.doi.org/10.1172/JCI40543

Lu J., Kong X., Luo Ch., Li K. K. (2013): Application of epigenome-modifying small molecules in induced pluripotent stem cells. Med. Res. Rev. **33,** 790–822
http://dx.doi.org/10.1002/med.21265

Lu X., Zhao T. (2013): Clinical therapy using iPSCs: hopes and challenges. Genomics Proteomics Bioinformatics **11,** 294–298
http://dx.doi.org/10.1016/j.gpb.2013.09.002

Marchetto M. C., Brennand K. J., Boyer L. F., Gage F. H. (2011): Induced pluripotent stem cells (iPSCs) and neurological disease modeling: progress and promises. Hum. Mol. Genet. **20,** R109–115
http://dx.doi.org/10.1093/hmg/ddr336

Mattis V. B., Svendsen C. N. (2011): Induced pluripotent stem cells: a new revolution for clinical neurology? Lancet. Neurol. **10,** 383–394
http://dx.doi.org/10.1016/S1474-4422(11)70022-9

Moretti A., Bellin M., Welling A., Jung C. B., Lam J. T., Bott-Flügel L., Dorn T., Goedel A., Höhnke C., Hofmann F., et al. (2010): Patient-specific induced pluripotent stem-cell models for long-QT syndrome. N. Engl. J. Med. **363,** 1397–1409 http://dx.doi.org/10.1056/NEJMoa0908679

Muangpaisan W., Mathews A., Hori H., Seidel D. (2011): A systematic review of the worldwide prevalence and incidence of Parkinson's disease. J. Med. Assoc. Thai. **94,** 749–755

Mummery C. L., Zhang J., Ng E. S., Elliott D. A., Elefanty A. G., Kamp T. J. (2012): Differentiation of human embryonic stem cells and induced pluripotent stem cells to cardiomyocytes: a methods overview. Circ. Res. **111,** 344–358 http://dx.doi.org/10.1161/CIRCRESAHA.110.227512

Miyoshi N., Ishii H., Nagano H., Haraguchi N., Dewi D. L., Kano Y., Nishikawa S., Tanemura M., Mimori K., Tanaka F., et al. (2011): Reprogramming of mouse and human cells to pluripotency using mature microRNAs. Cell Stem Cell **8,** 633–638 http://dx.doi.org/10.1016/j.stem.2011.05.001

Narsinh K., Narsinh K. H., Wu J. C. (2011): Derivation of induced pluripotent stem cells for cardiovascular disease modeling. Circ. Res. **108,** 1146–1156 http://dx.doi.org/10.1161/CIRCRESAHA.111.240374

Nelson T. J., Martinez-Fernandez A., Yamada S., Perez-Terzic C., Ikeda Y., Terzic A. (2010): Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. Circulation **120,** 408–416 http://dx.doi.org/10.1161/CIRCULATIONAHA.109.865154

Nguyen H. N., Byers B., Cord B., Shcheglovitov A., Byrne J., Gujar P., Kee K., Schüle B., Dolmetsch R. E., Langston W., Palmer T. D., Pera R. R. (2011): LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. Cell Stem Cell **8,** 267–280 http://dx.doi.org/10.1016/j.stem.2011.01.013

Nicholas C. R, Chen J., Tang Y., Southwell D. G., Chalmers N., Vogt D., Arnold C. M., Chen Y. J., Stanley E. G., Elefanty A. G., et al. (2013): Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development. Cell Stem Cell **12,** 573–586 http://dx.doi.org/10.1016/j.stem.2013.04.005

Norton S., Matthews F. E., Barnes D. E., Yaffe K., Brayne C. (2014): Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet Neurol. **13,** 788–794 http://dx.doi.org/10.1016/S1474-4422(14)70136-X

Nsair A., MacLellan W. R. (2011): Induced pluripotent stem cell for regenerative cardiovascilar therapies and biomedical discovery. Adv. Drug Deliv. Rev. **63,** 324–330 http://dx.doi.org/10.1016/j.addr.2011.01.013

Oh Y., Wei H., Ma D., Sun X., Liew R. (2012): Clinical application of patientspecific induced pluripotent stem cells in cardiovascular medicine. Heart **98,** 443–449 http://dx.doi.org/10.1136/heartjnl-2011-301317

Okano H., Yamanaka S. (2014): iPS cell Technologies: significance and applications to CNS regeneration and disease. Mol. Brain **7,** 22 http://dx.doi.org/10.1186/1756-6606-7-22

Okita K., Nagata N., Hyenjong H., Ichisaka T., Yamanaka S. (2008): Generation of mouse induced pluripotent stem cells without viral vectors. Science **322,** 949–953 http://dx.doi.org/10.1126/science.1164270

Onder T. T., Daley G. Q. (2012): New lessons learned from disease modeling with induced pluripotent stem cells. Curr. Opin. Genet. Dev. **22,** 500–508 http://dx.doi.org/10.1016/j.gde.2012.05.005

Payne N. L., Sylvain A., O'Brien C., Herszfeld D., Sun G., Bernard C. C. A. (2015): Application of human induced pluripotent stem cells for modeling and treating neurodegenerative diseases. N. Biotechnol. **32,** 212–228 http://dx.doi.org/10.1016/j.nbt.2014.05.001

Pu J., Hiang H., Zhang B., Feng J. (2012): Redefining Parkinson's disease research using induced pluripotent stem cells. Curr. Neurol. Neurosci. Rep. **12,** 392–398 http://dx.doi.org/10.1007/s11910-012-0288-1

Reinhardt P., Schmid B., Burbulla L. F., Schöndorf D. C., Wagner L., Glatza M., Höing S., Hargus G., Heck S. A., Dhingra A., et al. (2011): Genetic correction of a LRRK2 mutation in human iPSCs links parkinsonian neurodegeneration to ERK-dependent changes in gene expression. Cell Stem Cell **12,** 354–367 http://dx.doi.org/10.1016/j.stem.2013.01.008

Salewski R. P. F., Eftekharpour E., Fehlings M. G. (2010): Are induced pluripotent stem cells the future of cell-based regenerative therapies for spinal cord injury? J. Cell. Physiol. **222,** 515–521

Seifinejad A., Tabebordbar M., Baharvand H., Boyer L. A., Salekdeh G. H. (2010): Progress and promise towards safe induced pluripotent stem cells for therapy. Stem Cell Rev. **6,** 297–306 http://dx.doi.org/10.1007/s12015-010-9121-x

Singh V. K., Kalsan M., Kumar N., Saini A., Chandra R. (2015): Induced pluripotent stem cells: applications in regenerative medicine, disease modeling, and drug discovery. Front. Cell Dev. Biol. **3,** 1–18 http://dx.doi.org/10.3389/fcell.2015.00002

Soldner F., Hockemeyer D., Beard C., Gao Q., Bell G. W., Cook E. G., Hargus G., Blak A., Cooper O., Mitalipova M., Isacson O., Jaenisch R. (2009): Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell **136,** 964–977 http://dx.doi.org/10.1016/j.cell.2009.02.013

Stadtfeld M., Nagaya M., Utikal J., Weir G., Hochedlinger K. (2008): Induced pluripotent stem cells generated without viral integration. Science **322,** 945–949 http://dx.doi.org/10.1126/science.1162494

Sundberg M., Bogetofte H., Lawson T., Jansson J., Smith G., Astradsson A., Moore M., Osborn T., Cooper O., Spealman R., Hallett P., Isacson O. (2013): Improved cell therapy protocols for Parkinson's disease based on differentiation efficiency and safety of hESC-, hiPSC-, and non-human primate iPSC-derived dopaminergic neurons. Stem Cells **31,** 1548–1562 http://dx.doi.org/10.1002/stem.1415

Takahashi K., Yamanaka S. (2006): Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell **126,** 663–676 http://dx.doi.org/10.1016/j.cell.2006.07.024

Takahashi K., Tanabe K., Ohnuki M., Narita M., Ichisaka T., Tomoda K., Yamanaka S. (2007): Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell **131,** 861–872

http://dx.doi.org/10.1016/j.cell.2007.11.019
Takahashi K., Yamanaka S. (2013): Induced pluripotent stem cells in medicine and biology. Development **140,** 2457–2461 http://dx.doi.org/10.1242/dev.092551
Tanabe K., Takahashi K., Yamanaka S. (2014): Induction of pluripotency by defined factors. Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. **90,** 83–96 http://dx.doi.org/10.2183/pjab.90.83
Thorrez L., Sampaolesi M. (2011): The future of induced pluripotent stem cells for cardiac therapy and drug development. Curr. Pharm. Des. **17,** 3258–3270 http://dx.doi.org/10.2174/138161211797904154
Vidarsson H., Hyller J., Sartipy P. (2010): Differentiation of human embryonic stem cells to cardiomyocytes for in vitro and in vivo applications. Stem Cell Rev. **6,** 108–120 http://dx.doi.org/10.1007/s12015-010-9113-x
Wang S., Bates J., Li X., Schanz S., Chandler-Militello D., Levine C., Maherali N., Studer L., Hochedlinger K., Windrem M., Goldman S. A. (2013): Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. Cell Stem Cell **12,** 252–264 http://dx.doi.org/10.1016/j.stem.2012.12.002
Wang Y., Hu J., Jiao J., Liu Z., Zhou Z., Zhao C., Chang L. J., Chen Y. E., Ma P. X., Yang B. (2014): Engineering vascular tissue with functional smooth muscle cells derived from human iPS cells and nanofibrous scaffolds. Biomaterials **35,** 8960–8969 http://dx.doi.org/10.1016/j.biomaterials.2014.07.011
Wanjare M., Kuo F., Gerecht S. (2013): Derivation and maturation of synthetic and contractile vascular smooth muscle cells from human pluripotent stem cells. Cardiovasc. Res. **97,** 321–330 http://dx.doi.org/10.1093/cvr/cvs315
Wernig M., Zhao J. P., Hedlund E., Fu D., Soldner F., Broccoli V., Constantine-Paton M., Isacson O., Jaenisch R. (2008): Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc. Natl. Acad. Sci. USA **105,** 5856–5861 http://dx.doi.org/10.1073/pnas.0801677105
Woltjen K., Michael I. P., Mohseni P., Desai R., Mileikovsky M., Hämäläinen R., Cowling R., Wang W., Liu P., Gertsenstein M., Kaji K., Sung H. K., Nagy A. (2009): PiggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature **458,** 766–770 http://dx.doi.org/10.1038/nature07863
Wong G. K. Y., Chiu A. T. (2011): Gene therapy, gene targeting and induced pluripotent stem cells: Applications in monogenic disease treatment. Biotechnol. Adv. **29,** 1–10 http://dx.doi.org/10.1016/j.biotechadv.2010.07.005
Yagi T., Ito D., Okada Y., Akamatsu W., Nihei Y., Yoshizaki T., Yamanaka S., Okano H., Suzuki N. (2011): Modeling familial Alzheimer's disease with induced pluripotent stem cells. Hum. Mol. Genet. **20,** 4530–4539 http://dx.doi.org/10.1093/hmg/ddr394
Yazawa M., Dolmetsch R. E. (2013): Modeling Timothy syndrome with iPS cells. J. Cardiovasc. Transl. Res. **6,** 1–9 http://dx.doi.org/10.1007/s12265-012-9444-x
Yoshida I., Yamanaka S. (2010): Recent stem cell advances: induced pluripotent stem cells for disease modeling and stem cell-based regeneration. Circulation **122,** 80–87 http://dx.doi.org/10.1161/CIRCULATIONAHA.109.881433
Young W., D'Souza S. L., Lemischka I. R., Schaniel C. (2012): Patient-specific induced pluripotent stem cells as a platform for disease modeling, drug discovery and precision personalized medicine. J. Stem Cell Res. Ther. S10, 1–14 http://dx.doi.org/10.4172/2157-7633.s10-010
Yu J., Hu K., Smuga-Otto K., Tian S., Stewart R., Slukvin I. I., Thomson J. A. (2009): Human induced pluripotent stem cells free of vector and transgene sequences. Science **324,** 797–801 http://dx.doi.org/10.1126/science.1172482

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