

Invited Review

Induced pluripotent stem cells and neurological disease models

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Abstract: The availability of human stem cells heralds a new era for *in vitro* cell-based modeling of neurodevelopmental and neurodegenerative diseases. Adding to the excitement is the discovery that somatic cells of patients can be reprogrammed to a pluripotent state from which neural lineage cells that carry the disease genotype can be derived. These *in vitro* cell-based models of neurological diseases hold promise for monitoring of disease initiation and progression, and for testing of new drug treatments on the patient-derived cells. In this review, we focus on the prospective applications of different stem cell types for disease modeling and drug screening. We also highlight how the availability of patient-specific induced pluripotent stem cells (iPS cells) offers a unique opportunity for studying and modeling human neurodevelopmental and neurodegenerative diseases *in vitro* and for testing small molecules or other potential therapies for these disorders. Finally, the limitations of this technology from the standpoint of reprogramming efficiency and therapeutic safety are discussed.

Key words: embryonic stem cells; induced pluripotent stem cells; neurological diseases; disease modeling; drug screening

诱导性多能干细胞与神经系统疾病模型的构建

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摘要: 利用外源性转录因子将已分化成熟的细胞诱导为多能干细胞(induced pluripotent stem cells, iPS细胞)对构建神经发育性和神经退行性疾病的体外模型具有重要意义。从患有特定疾病或具有明确遗传基因异常的患者身上获取体细胞进行诱导建立的iPS细胞具有潜在的疾病原特质, 这种疾病特异性iPS细胞可以用来研究疾病的发病机制和病理过程, 也为药物筛选、药物毒性检测以及个体化治疗方案的制定提供了可能。本文概述了目前用于建立神经系统疾病模型的干细胞类型, 重点讨论了利用iPS细胞技术建立脊髓性肌萎缩症、肌萎缩性脊髓侧索硬化症和帕金森病等常见中枢神经系统疾病模型和药物干预的最新进展, 并对该研究领域所面临的问题进行了详尽的分析。

关键词: 胚胎干细胞; 诱导性多能干细胞; 神经系统疾病; 疾病模型; 药物筛选

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1 Making use of stem cells in neurological diseases

The central nervous system has limited capacity for regeneration in acute injuries, such as stroke and spinal

cord injury and more so under degenerative conditions, such as in amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), Parkinson's disease (PD) and Huntington's disease (HD) [1]. Although the adult brain contains a small number of stem cells in restricted

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areas, these stem cells do not contribute significantly to functional recovery. Transplantation of stem cells or stem cell-derived progenitors has long been taken as a therapeutic strategy to repair the damaged brain. Animal experiments have shown that replacement of the target neural tissue with stem cells derived from various sources can lead to recovery of lost function [2]. In humans, however, trials of transplantation after the onset of early symptoms yielded diverse outcomes, ranging from significant clinical benefit to worsening of symptoms and severe side effects [1]. As the pathophysiology differs among various neurological disorders, future therapy and/or drug screening must focus on the use of specific disease models to delineate the mechanism of pathogenic progression for the design of therapeutic strategies.

Given that neurons within the central nervous system are rarely sampled during the course of human diseases for neuropathological studies, our current knowledge of human disease-related neuronal phenotypes are based largely on analyses of postmortem brain tissues that at best represent late phases of the disease. Although transgenic/gene-knockout mouse models provide means to mimic genetic forms of neurodegenerative diseases in humans, this approach is limited to monogenic disorders and thus can only represent a minority of diseases. Species differences present another challenge for this technology. This indicates the need for advancement toward humanized models [3]. In the mean time, the recent developments in human pluripotent stem cell technology has provided renewed impetus to the derivation of neural cell lineages that harbor disease-specific genetic lesions for *in vitro* modeling of disease and screening for drug safety [4].

2 Human stem cells for neurological disease modeling

2.1 Embryonic stem (ES) cells

The inner cell mass of mammalian embryos in the blastocyst stage is a source of pluripotent stem cells [5]. These ES cells are capable of unlimited self-renewal [4] and have the capacity to differentiate into all somatic cell types of the body, including cells of the neural lineage [6]. Of particular interest is the opportunity to derive human ES cells from blastocysts left over from preimplantation genetic diagnosis (PGD) programmes. These cells either carry congenital mutations for specif-

ic disease states or can be subjected to genetic manipulation that model disease-specific aberrations and thus become available for *in vitro* modeling [4]. Such *in vitro* models will complement studies based on animal models of the diseases. PGD-derived human ES cells have been developed to model such CNS-related disorders as HD [7], fragile X syndrome [8], and ALS. These PGD-cell lines provide a valuable source of undifferentiated and differentiating ES cells as well as derivative neurons, glia, and other somatic cells for investigations of the cause, effect, and treatment at the cell/tissue level. Recently, an *in vitro* model of familial type of ALS (fALS) was established from human ES cells with a (G93A) mutation in the SOD1 gene. This model mimics the human ALS disease in terms of selective degeneration of spinal motor neurons that express the SOD1 (G93A) mutation [9]. Similarly, a closely representative cell model of SMA was achieved with PGD-derived human ES cells whereby knocking down the disease-determining gene improved survival of resulting motor neurons [10]. Otherwise, the affected spinal motor neurons was significantly impaired and subsequently degenerated. Administration of antioxidants prevented disease-related apoptosis of the motor neurons, highlighting the importance of antioxidants for the treatment of SMA. A human ES cell-derived neuronal model of HD was also reported [11]. The HD lines exhibited cytotoxicity tied to levels of soluble, monomeric forms of the mutant huntingtin protein (Htt) but not to those of aggregated forms presenting as neuronal inclusion bodies. Consistent with Rhes-mediated stabilization of mutant Htt monomers, knockdown of *Rhes* protected the HD lines against mutant Htt toxicity. Therefore, human ES cell-derived neuronal models are not only useful for unravelling molecular mechanisms of neurological diseases, but also bring new possibilities to the discovery of drugs that interfere with key steps in the neurodegenerative process. Despite the potential for translational medicine, interest has been tempered by ethical concerns and legal restrictions for deriving and using stem cell lines from human blastocysts [12].

2.2 Adult stem (AS) cells

In contrast to ES cells, AS cells reside in specialized niches of mature tissues [4]. The AS cells fulfill the basic criteria of stemness, having the capacity to self-renew and to give rise to one or more differentiated cell types of the tissue in which they reside. In addition,

many AS cells show plasticity to differentiate into cell types other than those of the expected lineages. Accessibility and abundance of AS cells aside, clearance from ethical issues is one other consideration that has focused research efforts on AS cells as viable alternative cell models for the study of human diseases of the central nervous system as well as for drug discovery.

For disease modeling, human AS cells from a healthy donor can be subjected to targeted gene manipulation or relevant cells can be isolated from a patient for generation of a disease-specific cell line [4]. Potential cell sources for disease modeling include mesenchymal stem cells (MSCs) and neural stem cells (NSCs). MSCs are proven to be superior to NSCs as they can be derived from a variety of adult tissues such as bone marrow [13–15] as well as other mesoderm-derived tissues. Although disease-specific MSCs have been derived from patients of PD, ALS and familial dysautonomia [16], no significant differences between healthy donors or patients were observed in terms of the biological properties of the MSCs and the characteristics of the derived neurons [17, 18]. NSCs recovered from the developing central nervous system are capable of proliferation and the cell progeny can be induced to differentiate into neurons and glial cells. Disease-specific or genetically engineered NSC lines also represent cellular systems for discerning disease etiology and drug candidates. For example, the emerging theory on the relevance of neurogenesis in neurodevelopmental disorders such as schizophrenia and autism is bolstering the use of NSCs as research tools [19]. While human NSC cultures are considered to be a gold standard for modeling neurological disease, limited access to tissues at source impedes the use of NSCs for disease modeling. Neither surgical nor post-mortem removal of brain tissues can provide sufficient biomaterial for large-scale cell-based drug screening.

2.3 Adult somatic cells

Recent work supported the possibility of reprogramming adult somatic cells into mature neurons without intermediary derivation of induced pluripotent stem (iPS) cells [20–22]. Wernig *et al.* tested a number of helix-loop-helix transcription factors that are expressed during neuronal development [23] and found that *ascl1* combined with other factors could induce reprogramming of mice fibroblasts into mature neurons. Pfisterer *et al.* demonstrated that by overexpression of the three transcription factors *Ascl1*, *Brn2*, and *Myt1l* with two other involved in dopamine neuron generation, *Lmx1a* and

FoxA2, human fibroblasts were reprogrammed into dopaminergic neurons [24]. Caiazzo *et al.* utilized a minimum set of but three transcription factors *Ascl1*, *Nr4a2* and *Lmx1a* to achieve the generation of dopaminergic neurons from fibroblasts [25]. Most recently, two small molecules (forskolin and dorsomorphin) enabled the transcription factor Neurogenin 2 (NGN2) or SOX11 to reprogram human fibroblasts into cholinergic neurons [26]. Son *et al.* reported that the forced expression of select transcription factors induced the reprogramming of fibroblasts into motor neurons. These motor neurons showed expected homing into the ventral horn of the spinal cord of the chick embryo after transplantation [27]. Similar success was reported for the reprogramming of skin fibroblasts harvested from an AD patient into functional neurons [28]. Recent reports demonstrated that transient induction of the four pluripotency factors (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*) followed by exposure to sonic hedgehog and fibroblast growth factor-8 was sufficient to reprogram fibroblasts to such progenitor cell types as induced NSCs and dopaminergic neuronal progenitors [29–31]. Taken together, these studies provide proof-of-principle that transcription factor-mediated reprogramming of human fibroblasts into subtype-specific neurons can be accomplished without passing through a proliferative progenitor state and therefore is ethically acceptable and minimally at risk of tumor formation.

2.4 iPS cells

A novel approach for stem cell generation is the attempt to induce differentiated somatic cells into pluripotent stem cells by introducing factors that can induce reprogramming of the cells [32, 33]. These iPS cells can be generated without the use of oocytes or cells from the preimplantation embryo, and thus bypass ethical issues that have limited the use of human ES cells [34]. Additionally, iPS cells can be derived from the patient who needs treatment, thereby overcoming problems of immune rejection associated with the use of allogeneic human ES cell-derived progenitors. Thus, advancements in iPS cell technology hold promise for patient-specific model systems that allow studies into the pathogenesis of disease and tests for effectiveness of pharmacological agents, as well as provision of ample sources of autologous cells for use in transplantation therapy [35].

3 Cell-based models of neurological diseases

One important aspect of iPS cell technology is the pos-

sibility to develop autologous cells for cell replacement therapy. The patient-specific pluripotent cells could be differentiated into desired cell types in unlimited cell numbers which ultimately could be transplanted into the patient for replacement of the affected tissue [36]. The possible generation of pluripotent cells from patients with developmental or degenerative disorder further allows for disease modeling and opens up new opportunities for drug discovery (Fig. 1). In addition, with the possibility of a bank of iPS cells derived from healthy donors of different ages, ethnic origin and sex [34], we can expect high-throughput drug screening for toxicology and safety to reduce the risks associated with research on human subjects.

3.1 SMA

SMA is one of the most common inherited forms of neurodevelopmental disease leading to infant mortality. SMA is caused by deletion or defect in the SMN1 gene, leading to deficiency of the SMN (survival of motor neuron) protein, dysfunction and death of spinal motor neurons, severe SMA and even death of the individual [37, 38]. The human SMN locus consists of a telomeric copy

(SMN1) and an inverted centromeric copy (SMN2) [39, 40], both with identical protein-encoding capacity. Despite the ubiquitous and indispensable function of SMNs, the selective motor neuron pathology remains unclear. Since only human has the two SMN isoforms, animal models are less than adequate for studies into the mechanism of motor neuron degeneration in the context of human SMN deficiency [41]. iPS cells derived from SMA patients could then be a source of unique cell models for dissecting the roles of different SMN isoforms in SMA. In a landmark study, Ebert *et al.* found that motor neurons that were derived from iPS cells of an SMA patient became progressively smaller and less numerous in culture than motor neurons derived from a healthy family member, thus recapitulating the disease *in vitro* [38]. With the addition of valproic acid and tobramycin, which are known to boost SMN production, the diseased cells in this model produced up to three times as much SMN protein as their untreated counterparts. These iPS cells from the SMA patient, however, showed reduced capacity to become mature motor neurons *in vitro* [38], which may be attrib-

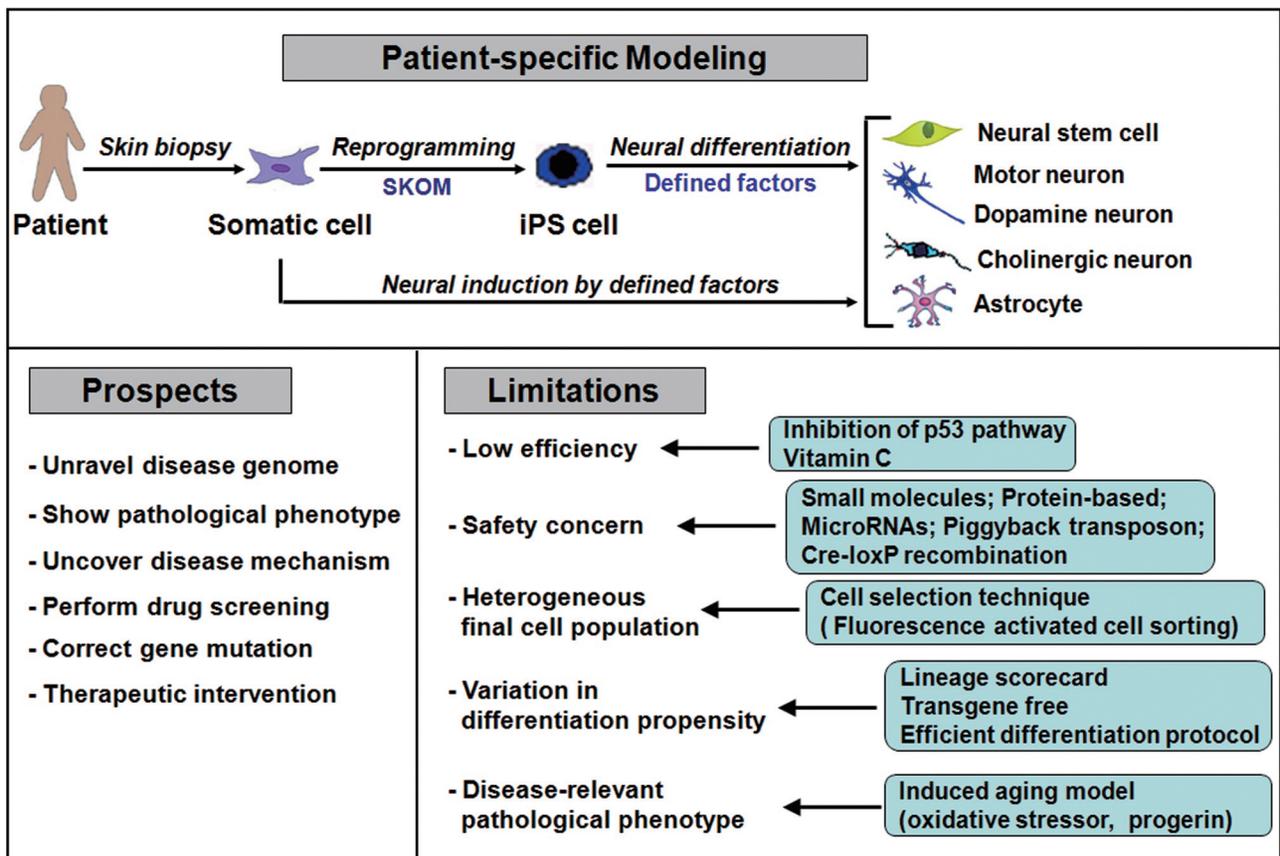


Fig. 1. Overview of the application of iPS cell-based model for human neurological diseases. SKOM: Sox2, Klf4, Oct4, and c-Myc.

uted to clonal variation rather than the underlying genetic defect. Thus, it is necessary to establish iPS cell lines from other SMA patients with similar phenotypes. Chang *et al.* reported the establishment of five iPS cell lines from a second type 1 SMA patient. These iPS cell lines exhibited reduced motor neuron production and slower neurite growth. To restore SMN expression, SMA iPS cells were transduced with HIV7/ SMN1, and ectopic SMN expression in these iPS cell lines restored normal motor neuron differentiation^[42]. These studies documented how a promising resource can be established to study disease mechanisms, screen new candidate drugs and develop new therapies.

3.2 ALS

ALS, one of the neurodegenerative diseases, is characterized by the degeneration of upper and lower motor neurons, leading to fatal paralysis^[43]. About 10% of ALS cases are familial (fALS) and 90% are sporadic (sALS) with largely unknown genetic etiology^[44]. fALS have been unambiguously associated with mutations in SOD1, DPP6, ITPR2, and TARDBP (also known as TDP-43)^[45]. A common theme in some forms of fALS—including mutant forms of SOD1 and TDP-43, is the formation of cytoplasmic aggregates^[46–48]. TDP-43 aggregates are found in the majority of sALS, implying that some mechanisms are common to the familial and sporadic forms. Initial studies reported that iPS cell-derived motor neurons from fALS patients with TDP-43 mutations showed not only cytoplasmic accumulation of TDP-43, but also decreased survival and altered neurite development *in vitro*^[49, 50]. However, both studies failed to validate their results with either a “rescue” approach or cohorts of sufficient size for statistical analysis. Recently, Burkhardt *et al.* demonstrated that iPS cell-induced motor neurons derived from three sALS patients displayed spontaneous intranuclear and hyperphosphorylated TDP-43 aggregates^[51]. From among a larger cohort with a total of 92 iPS cell clones, they also found similar intranuclear TDP-43 aggregates in postmortem brain and spinal cord tissue of the sALS patients, therefore directly connecting the phenotype in iPS cell-induced motor neurons back to the pathology. The model of ALS iPS cell-derived neurons showing *de novo* TDP-43 pathology would be invaluable for gaining insight into the biology of wild type TDP-43 aggregation and drug discovery.

The mutation in SOD1 results in classical inherited fALS. Most studies have focused on the mechanism of

SOD1-mediated motor neuron degeneration^[52–54]. In rodents, over-expression of mutated SOD1 causes phenotypes of an ALS-like motor neuron disease and this is not rescued by over-expression of wild-type SOD1^[55]. While several drugs have therapeutic effects in SOD1 transgenic rodents, clinical trials in humans have not been successful^[56], suggesting that current ALS rodent models are unable to provide full insight into the pathogenesis of human ALS. Human ES cells have been used for modeling both the autonomous and the non-cell-autonomous effects of ALS *in vitro*, using the mutated SOD1 gene^[3, 57, 58]. iPS cells have the same advantages as ES cells^[43]. In 2008, Dimos *et al.* successfully generated iPS cells from skin samples obtained from two elderly fALS patients displaying a mutation in SOD1^[59]. These iPS cells could differentiate into disease-free motor neurons and glial cells. Although a cohort of iPS cell-derived motor neurons with ALS-associated mutations in SOD1 has been generated, a phenotypic analysis with respect to disease-relevant properties and features will be of interest^[60]. Therefore, it remains unclear if neurons derived from iPS cells have the potential to recapitulate late-onset pathology of ALS *in vitro*.

3.3 PD

PD is the second most common neurodegenerative disease. Prominent clinical features are motor symptoms (bradykinesia, tremor, rigidity, and postural instability) and non-motor symptoms (olfactory deficits, autonomic dysfunction, depression, and sleep disorders)^[3]. Loss of dopaminergic neurons in the substantia nigra of the basal ganglia is a characteristic neuropathological hallmark^[20]. Many genes have been directly associated with PD (PARK2, SNCA, UCHL1, LRRK2, PARK7, PINK1, GBA, and SNCAIP), although most of PD cases appear to be sporadic^[61]. Given that ES cells can be differentiated into the dopamine neurons, both mouse and human ES cell models of PD have been generated by over-expression of the mouse Nr4a2 and the human mutant α -synuclein^[62–65]. However, ES cell-established disease model has limitations. For example, none of these lines had the genetic background of a PD patient. Preimplantation genetic testing of embryos during selection for *in vitro* fertilization is only available for cases with single mutations. Thus, acquisition of sporadic PD ES cells by this method would be very difficult because in most cases the genetic factors are unknown^[45].

Generation of iPS cells from patients with PD has recently been described [66–68]. None of them was initially reported for the PD-specific iPS cells. However, evidence was provided for iPS cells with a mutation in PINK1 in that they differentiated into dopaminergic neurons which upon mitochondrial depolarization showed impaired recruitment of lentivirally expressed PARK2 to mitochondria and increased mitochondrial copy number [67]. Environmental insults such as toxins have been found to interact with genetic factors in the pathogenesis of PD. Autosomal-dominant mutations in LRRK2 encoding a large multidomain kinase is the most common known familial genetic cause of PD. LRRK2 mutant iPS cell-derived neurons from familial PD patients have been associated with increased sensitivity to oxidative stress including 6-hydroxydopamine or 1-methyl-4-phenylpyridinium—which selectively enter dopaminergic neurons through the dopamine transporter—as well as hydrogen peroxide or rotenone [69–71]. Similarly, iPS cell-derived neurons that harbor PD-associated homozygous recessive mutations in PINK1, a mitochondrial kinase, or a familial inherited triplication of the α Syn locus have showed increased sensitivity to oxidative toxins [69]. In addition, studies in human iPS cell-derived neuronal models of PD have also sought to reveal the role of mitochondrial alterations in the PD pathogenesis. iPS cell-derived neurons with mutations in PINK1 have been reported to display mitochondrial function abnormalities, defective mitochondrial quality control, and altered recruitment to mitochondria of exogenously transduced PARKIN, a ubiquitin ligase encoded by another familial PD gene [72]. Surprisingly, PARKIN-deficient iPS cell-derived neurons from familial PD patients did not appear to show frank mitochondrial defects, suggesting potential redundancy [73]. Another prominent feature of PD pathology is the accumulation of α Syn protein. Heterozygous carriers of mutations in β -glucocerebrosidase (GBA), which encodes an essential lysosomal degradation machinery enzyme, are at increased risk of PD, and iPS cell-derived neurons from such individuals was shown to display a dramatically increased accumulation of α Syn protein [74]. As expected, iPS cell-derived neurons harboring triplication of the α Syn locus revealed similarly increased accumulation of α Syn protein [75, 76]. Chung *et al.* discovered a small molecule (NAB2) and its downstream molecule (ubiquitin ligase Nedd4) reversed pathologic phenotypes in iPS cell-

derived neurons from PD patients with α Syn mutation [77]. iPS cell-induced neurons derived from PD patients showed a loss of particular age-associated features [75, 77], indicating that iPS cell-based studies failed to recapitulate the pathophysiology of late onset neurodegenerative diseases. Most recently, Miller *et al.* amazingly developed a method to overcome these limitations and successfully modeled age-dependent neurodegeneration using iPS cell-derived neurons [78]. They induced the overexpression of progerin, a truncated form of lamin A which is associated with premature aging, in PD iPS cell-derived neurons and established induced aging in these neurons. The induced neurons revealed disease phenotypes which include marked dendrite degeneration (a progressive loss of tyrosine hydroxylase expression), enlarged mitochondria or Lewy-body-precursor inclusions, all being features associated with genetical susceptibility and aging. In this respect, iPS cell-based studies of age-dependent neurodegeneration can reveal features that manifest late in disease progression, and thus complement those that reflect disease predisposition.

3.4 Others

Thus far, in addition to the aforementioned models, various iPS cell lines from patients with such neurodegenerative disorders as AD, HD, familial dysautonomia, Rett's syndrome, and Down's syndrome have also been generated. Interested readers can refer to recent in-depth studies on the respective disorders [79].

4 Challenges in iPS cell-based disease modeling

One limitation of iPS cell technology is the poor efficiency in derivation of iPS cells, as accounted for by elite and stochastic models of the reprogramming mechanism [80, 81]. The stochastic model predicts that cellular senescence is a major obstacle to the generation of iPS cells. Suppression of the p53 pathway involved in the senescence regulator network should promote the generation of iPS cells and shorten the time for reprogramming. Recent studies show that vitamin C enhances iPS cell generation at least in part by suppressing the senescence regulator Ink4a/Arf [82]. Small molecules, including DNA methyltransferase inhibitors and the histone deacetylase inhibitor, valproic acid, have also been successfully used as substitutes for the induction by forced expression of transcription

factors [83, 84]. The mechanism by which these molecules facilitate reprogramming however remains unclear.

In order to use iPS cells for treating human disease, it is necessary to assess the safety of the cells before clinical applications. In general, retro- or lentiviral transduction systems are used to generate iPS cells [85]. This virus-mediated transgene integration may lead to mutagenesis at the insertion site or altered expression of surrounding genes so that cellular dysfunction or tumorigenesis may result. To avoid this, nonintegrative approaches, such as the use of adenoviruses or repeated transfection with plasmid vectors, episomal vectors and piggyback transposons were attempted with some success [86, 87]. Kim *et al.* generated fusion proteins in which each of the four reprogramming factors was fused to a cell-penetrating peptide sequence, thereby enabling it to cross the cellular membrane [88]. The protein-based iPS cells were differentiated into dopaminergic neurons [89], which showed similar properties to midbrain dopaminergic neurons *in vitro* and could rescue motor deficits when transplanted into PD rat models. However, these methods may require repeated rounds of treatment and suffer from reprogramming efficiencies that are up to 1 000-fold lower than with retroviral vectors [35].

Although there is a large degree of similarity in both gene expression and epigenetic marks among iPS cell and ES cell lines [90], iPS cells are not identical to ES cells. Concerns stem from the fact that iPS cells retain gene expression pattern of the cell type of origin and thus remain different from ES cells [90]. Variability may also be attributed to the introduction of reprogramming factors with use of randomly integrating viral vectors [91–93]. Quantitative studies of stem cell differentiation support the view that each line differs in propensity for differentiation down a given lineage, including neural cells [60, 94]. This discordant behavior could cause substantial problems in attempts to establish neurological disease models. For example, many stem cell lines appear to be restricted from the neural lineage, thus resulting in low efficiency in the production of neural progenitors and postmitotic neurons [60]. To address this dilemma, high-throughput characterization of pluripotent cell lines for differentiation propensity resulted in establishment of a “lineage scorecard” [95]. This approach could serve to accurately predict lines which are most useful for producing neurons for disease-related studies.

Another hurdle to be overcome before patient-specific iPS cells can be applied clinically is the identification

of phenotypes characteristic of late onset neurological disorders, such as PD and HD. One possibility to address this challenge would be to accelerate aging of fully differentiated neural lineage cells and to assess the pathological phenotypes following exposure of the “aged” cell types to oxidative stressors, hydrogen peroxide, or MG-132 [66, 70, 96]. In Miller’s work, overexpression of progerin in the PD-iPS cell-derived dopaminergic neurons resulted in disease-related phenotypes that significantly and specifically mimicked those of late-onset PD [97]. This breakthrough will impact on studies of other neurodegenerative diseases with long latency. In the future, immediate development of high-throughput screening platforms is expected to be set up for the development of novel therapeutic interventions that will prove clinically relevant after the onset of symptoms in patients.

5 Conclusion

iPS cell-based disease models are providing valuable insight into the pathogenesis of human neurological diseases and cellular targets of therapeutic intervention. This strategy, however, is still in its infancy. The ultimate goal of iPS cell-based disease modeling is to generate personalized iPS cells so that the patient-specific cell model can be used to validate a drug treatment strategy for each patient. Firstly, the transgene-free iPS cells should be produced in order to minimize or eliminate genetic alterations in the derived iPS cell lines. It has been reported that the gene expression features of factor-free PD-iPS cells were closely similar to ES cells [36, 66]. Secondly, the efficiency of neural differentiation seems to be lower and more variable in iPS cell lines than in ES lines [90]. This is not affected by the source of fibroblasts, age, choice of reprogramming vectors, or residual transgene expression and may partially be attributed to variable response to neural inducers [98] and possible incomplete repression of fibroblast gene expression. Thus, refinement of differentiation protocols will be needed. Another challenge is the inability to select a desired cell population for expansion without contamination by other cell types. This raises the need for an efficient selection technique for purifying the disease-related cell types for further investigation. Lastly, disease-relevant phenotypes must be observable under *in vitro* conditions that sustain the viability of the patient-specific cell model. Progerin-

induced aging holds promise as means to advance the emergence of late onset complex disease-related phenotypes and thus widen the perspective of human iPS cell-based models of neurodegenerative disease.

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