Induced pluripotent stem cells: a new revolution for clinical neurology?

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Why specific neuronal populations are uniquely susceptible in neurodegenerative diseases remains a mystery. Brain tissue samples from patients are rarely available for testing, and animal models frequently do not recapitulate all features of a specific disorder; therefore, pathophysiological investigations are difficult. An exciting new avenue for neurological research and drug development is the discovery that patients' somatic cells can be reprogrammed to a pluripotent state; these cells are known as induced pluripotent stem cells. Once pluripotency is reinstated, cell colonies can be expanded and differentiated into specific neural populations. The availability of these cells enables the monitoring in vitro of temporal features of disease initiation and progression, and testing of new drug treatments on the patient's own cells. Hence, this swiftly growing area of research has the potential to contribute greatly to our understanding of the pathophysiology of neurodegenerative and neurodevelopmental diseases.

Lancet Neurol 2011; 10: 383–94

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Introduction

Neurodegenerative diseases are increasing in prevalence worldwide as the population ages. For some disorders, genomic studies have revealed underlying genetic mutations, but progress linking them to changes in neuronal function has been slow.[1] For instance, the genetic cause of Huntington's disease, an autosomal dominantly inherited neurological disorder, has been known since 1993; however, few new drugs have been developed after decades of research. In other neurological diseases, patients classified as having sporadic disease do not apparently carry any genetic mutations but might have developed the disorder through a combination of genetic and environmental factors. Hence, creation of accurate animal models of these diseases is difficult.

The technology that enables generation of induced pluripotent stem (iPS) cells is around 4 years old. While this specialty is still in its scientific infancy, it is rapidly evolving. In this Review we discuss how iPS cells might be used to fill the gaps in modelling of human neurological diseases by creating a novel approach known as "disease in a dish". The therapeutic potential of pluripotent stem cells has been described and will not be discussed here.[2–5] As with any new technology, however, caveats and hurdles hinder rapid introduction of this technology into the mainstream of modelling studies.

Induced pluripotent stem cells

The study of induced pluripotency has its roots in stem-cell biology and mammalian cloning. Primitive embryonic stem (ES) cells can be isolated from the inner cell mass of mouse, monkey, and human blastocysts. These cells may be expanded in culture while retaining pluripotency, or the ability to make all cells in the body.[6–8] Additionally, cloning of a sheep from an adult somatic cell showed that cells from mature mammalian tissue can be used to make a whole new organism.[9,10] In 2006, a landmark paper by Takahashi and Yamanaka[11] described how adult mouse fibroblasts could be reprogrammed back to a primitive state by overexpression of four genes expressed in ES cells: *Pou5f1*, *Sox2*, *Klf4*, and *cMyc* (figure 1). These reprogrammed cells, named iPS cells, were similar to ES cells and had the capacity to generate any cell in the body.[10] On the basis of these findings three independent groups successfully used similar genes to reprogramme human fibroblasts into iPS cells.[12–14]

ES cells are grown in small colonies in a two-dimensional culture system, and require a feeder layer (generally mouse embryonic fibroblasts [MEFs]) or a feeder-free synthetic substrate (generally matrigel) to survive (figure 2). Similarly

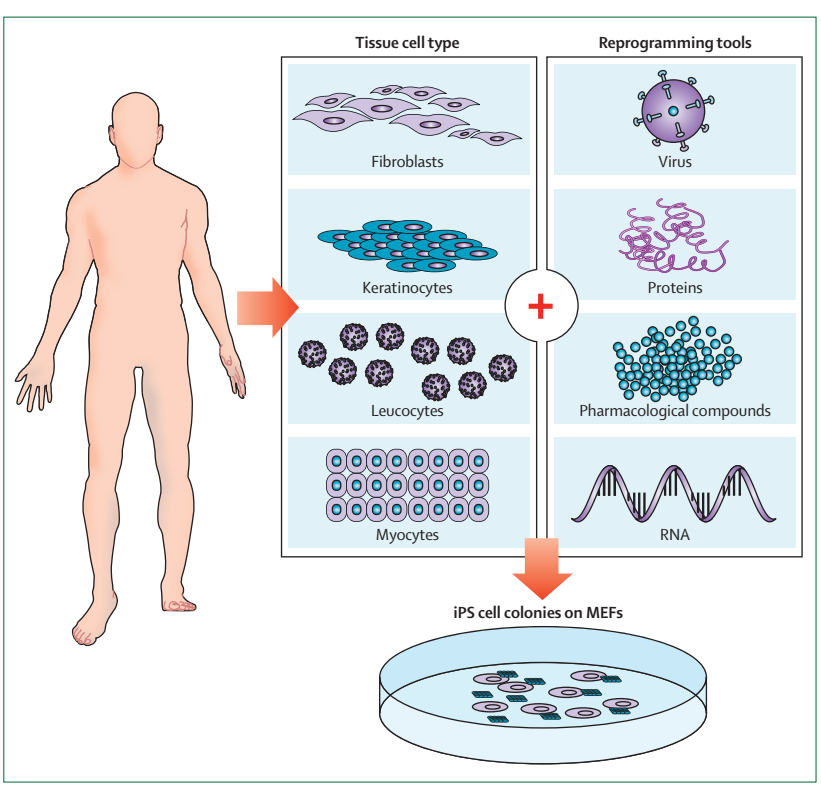

***Figure 1*: Generation of iPS cells**
Somatic cells (eg, fibroblasts, keratinocytes, leucocytes, or myocytes) are recovered from biopsy samples taken from a patient, and are reprogrammed to a pluripotent state. Reprogramming can be accomplished by use of a virus to transduce pluripotency genes, or with a combination of proteins, messenger RNAs, or various small molecules. Once reprogrammed, the cells are seeded on to MEFs (pink cells) and form colonies (blue cells). iPS=induced pluripotent stem. MEFs=mouse embryonic fibroblasts.

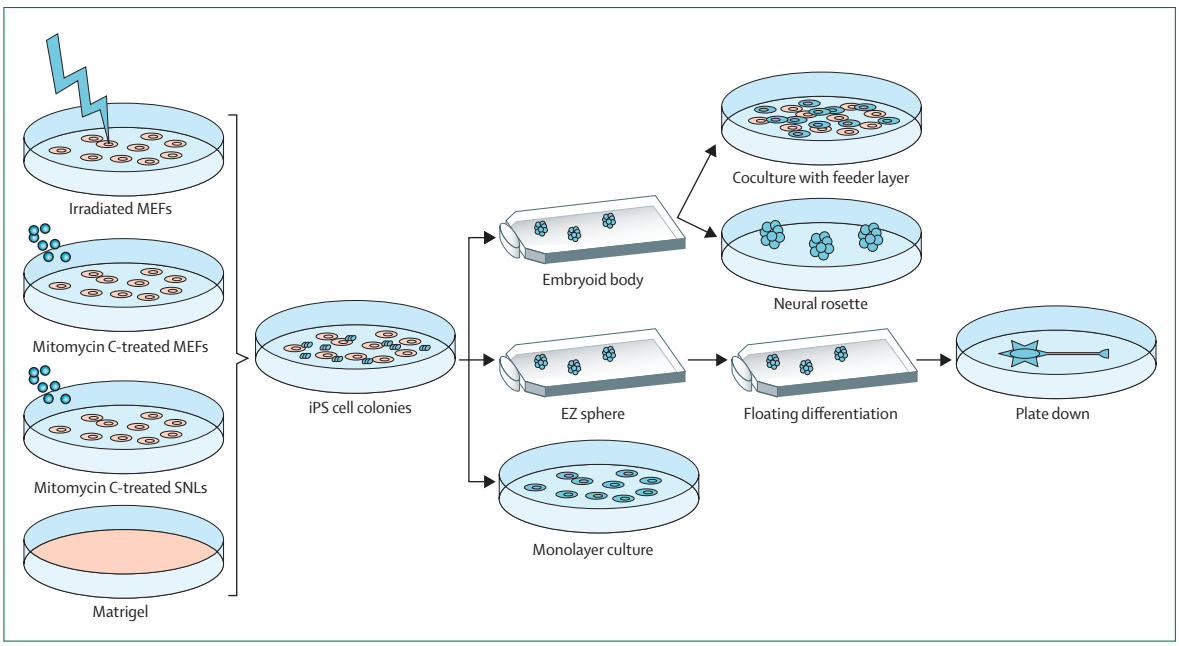

***Figure 2*: Methods of growth and neuronal induction of iPS cells**
Colonies of ES or iPS cells are grown in a two-dimensional culture system on a feeder layer, such as MEFs or SNL, or on a matrigel feeder-free layer. MEFs and SNLs are irradiated or treated with mitomycin C to prevent division. Neural induction can be performed in different ways, such as by using embryoid bodies, EZ spheres or monolayers. Further differentiatiation can be achieved by coculture with stromal feeder layers, neural rosette formation, or floating differentiation until plating. ES=embryonic stem. MEFs=mouse embryonic fibroblasts. SNLs=STO (MEF) fibroblasts expressing neomycin resistance and leukaemia inhibitory factor (LIF). iPS=induced pluripotent stem.

to ES cells, iPS cells form dense colonies, endogenously express pluripotency genes and embryonic cell surface antigens, have telomerase activity, and can form all three embryonic tissue types. iPS cells, however, have an important distinguishing feature, which is that they retain epigenetic memory from the source tissue.[15,16]

Modelling of neurological disease

Generation of fully accurate animal models of human neurological diseases has proved difficult. For instance, in Huntington's disease, for which the exact genetic deficit is well known, mouse models that closely mimic some features of disease progression (eg, the huntingtin protein N-terminal fragment models related to protein aggregation, reduced striatal volume, astrogliosis, and behavioural changes) do not match the true genetics, onset, and neuropathology of the disease in human beings.[1] Another example is that of Down's syndrome, which is characterised by a chromosome 21 trisomy, but as mice do not have a chromosome 21, disease models rely on the suboptimum search for homologous regions on different chromosomes.

Cellular models are used in addition to animal models to study neurological diseases. Non-neural samples taken from healthy tissues in patients with the disease, however, would not have all the phenotypic features of the disease. For instance, in patients with spinal muscular atrophy, the disease is characterised by decreased concentrations of the SMN protein in all tissues. Fibroblasts are readily available from patients, and hence these cells are used to screen for drugs that could increase the expression of this protein.[17–19] These cells are not, however, the neuronal cells affected by degeneration. Thus, although the ability of some drugs to raise protein concentrations in fibroblasts has been validated in animal models,[18,20] the pathology is not fully recapitulated and the effects in human beings might not be accurately represented.

To overcome the limitations of animal models, immortalised neural cells have been used in tissue culture models. For example, immortalised rat striatal cells that overexpress mutant *HTT*, which causes Huntington's disease, and a human neuroblastoma cell line that overexpresses *SNCA*, which is associated with Parkinson's disease, both show some of the cellular phenotypes detected in patients.[21,22] The use of immortalised cell lines from rodents is, however, not the ideal genetic background for testing drugs for human diseases. Furthermore, the immortalisation process might itself lead to abnormal cell physiology. Additionally, overexpression of known disease-causing genes creates an artificial system, as insertion into the genome is frequently random and under the control of an exogenous promoter. Finally, RNA splicing and other post-transcriptional or translational modifications occur according to the specific cellular background, and might not coincide with those processes in another cell type.

The use of iPS cells to create novel models of neurological disorders could be especially useful because these cell lines can be generated from patients and hence

they carry the actual mutations associated with the disease. 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 modelled disease phenotypes. Cell lines have been generated from tissues taken from both young and elderly patients, and that has permitted the study of early-onset[23] and late-onset[24] neurological disorders.

Once the iPS cell lines are generated, various differentiation pathways can be followed to derive the many types of neurons and neuronal support cells found in the brain and spinal cord. The protocols were

	Human ES cells	Monkey ES cells	Mouse ES cells	Human iPS cells	Mouse iPS cells
Retina cells					
Osakada et al[25]	Day 170: 19·6% (4·1%) of colonies *CRX*$^+$	Day 120: 24·0% (4·4%) of total cells CRX$^+$	Day 9: 15% of total cells RX-GFP$^+$ Day 20: 24·5% (1·6%) of RX-GFP$^+$ sorted cells CRX$^+$	..	..
Meyer et al[26]	Day 16: 95% of total cells *RAX*$^+$ Day 80: 19·4% (3·1%) of EZ spheres contained *CRX*$^+$ cells and within these 63·0% (7·6%) of all cells *CRX*$^+$	..	..	Day 80: 14·4% (5·1%) of spheres contained CRX$^+$ cells and within these 65·5% (9·3%) of all cells CRX$^+$	..
Striatal neurons					
Aubry et al[27]	Day 62–72: 22% (2%) of total cells *MAP2*$^+$ and of these 53% (6%) *PPP1R1B*	..	..	..	..
Dopaminergic					
Perrier et al[28]	Day 50: 30–50% of total cells tubulin β-III$^+$ and of these, 64–79% TH$^+$	..	..	..	..
Cooper et al[29]	Day 49: ~2% of total cells tubulin β-III$^+$/FOXA2$^+$/TH$^+$	..	..	Day 49: <1% of total cells tubulin β-III$^+$/FOXA2$^+$/TH$^+$	..
Motor neurons					
Li et al[30]	Day 49: ~21% of total cells HB9$^+$	..	..	..	..
Li et al[31]	Day 35: ~30–45% of total cells HB9$^+$	..	..	..	..
Ebert et al[23]	..	..	..	Day 28: 12·6 % (2·2%) and 9·5% (2·4%) of tubulin β-III$^+$ cells HB9$^+$	..
Dimos et al[24]	..	..	..	NS	..
Oligodendrocytes					
Izrael et al[32]	~Day 25–31: 42·5 (2·4%) of total cells O4$^+$ with ramified branches	..	..	..	..
Kang et al[33]	Stage V: ~81% of total cells O1$^+$	..	..	..	..
Nistor et al[34]	Day 49: 85% ± 5% of total cells O4$^+$	..	..	..	..
Keirstead et al[35]	Day 42: 83±7% of total cells O1$^+$	..	..	..	..
Tokumoto et al[36]	..	..	Day 19: 24·0% (6·0%) of total cells O4$^+$	..	Day 19: 2·3% (0·5%) O4$^+$
Miura et al[37]	..	..	NS		NS
Okada et al[38]	..	..	Day 5 (secondary neurospheres): 6·8% of total cells O4$^+$	..	..
Astrocytes					
Barberi et al[39]	..	..	Day 18: 92±5% of total cells GFAP$^+$	..	..
Miura et al[37]			NS		NS
Okada et al[38]	..	..	Day 5 (tertiary neurospheres): 0·7% of total cells GFAP$^+$	..	..
Peripheral neurons and oligodendrocytes					
Lee et al[40]	Day 28: 55% of colonies SMA$^+$ and 25% of colonies Peripherin$^+$	..	..	..	..
Pomp et al[41]	Day 28: 34·5% of colonies Peripherin$^+$/tubulin β-III$^+$	..	..	..	..
Cerebellar neurons					
Su et al[42]	Day 20: ~0·5% of total cells L7$^+$	..	..	..	..
Tao et al[43]	..	..	Day 20: 82·1% (10·5%) of total cells InsP3R1$^+$/ tubulin β-III$^+$	..	..

Data are duration of differentiation in days and mean (SD) proportion of cell type of interest generated. ES=embryonic stem. iPS=induced pluripotent stem. TH=tyrosine hydroxylase. NS=not stated.

Table 1: **Differentiation protocols to generate ES and iPS cells**

	Associated genes		iPS cells generated
	Leading to disease	Increasing susceptibility	
Neurodevelopmental disorders			
Single-gene mutation			
Angelman's syndrome	*UBE3A*	..	No
Rett's syndrome	*CDKL5*, *MECP2*	..	Yes[44]
Nucleotide repeat disorder			
Fragile X syndrome	*FRAXA*	..	Yes[45]
Chromosomal abnormalities			
Down's syndrome	Trisomy 21	..	Yes[14]
Prader-Willi syndrome	Paternal 15q11-13	..	Yes[46]
Cri du chat syndrome	5p15	..	No
Autism	..	*NLGN3*, *NHE9*, *SLC9A9*, *CNTNAP2*, *PCDH10*, *SCN7A*, *BZRAP1*, *MDGA2*	No
Neurodegenerative disorders			
Single-gene mutation			
Spinal muscular atrophy	*SMN1*	..	Yes[23]
Batten's disease	CLN3	..	No
Familial dysautonomia	*IKBKAP*	..	Yes[47]
Ataxias	*SPTBN2*, *TTBK2*, *PP2R2B*, *PP2R2B*, *PRKCG*, *ITPR1*, *PDYN*, *FGF14*	..	No
Nucleotide repeat disorder			No
Huntington's disease	*HTT*	..	Yes[14,48]
Spinocerebellar ataxia	*ATXN1*, *ATXN2*, *ATXN3*, *ATXN10*, *CACNA1A*	..	No
Friedreich's ataxia	*USP9X*	..	Yes[49]
Multigene or unknown origin			
ALS	*SOD1*, *VCP*, *ALS2*, *TARDBP* (also known as *TDP43*)	*DPP6*, *ITPR2*	Yes[24]
Retinal degeneration or disease	*CLRN1*, *USH1C*, *USH1G*, *USH2A*, *FZD4*, *LRP5*, *NDP*, *BEST1*, *PRPH2*, *VCAN*	*CDH23*, *GPR98*, *MYO7A*, *PCDH15*, *MT-ATP6*	No
Parkinson's disease	*PRKN*, *SNCA*, *LRRK2*, *PARK7*, *PINK1*,	*GBA*, *SNCAIP*, *UCHL1*	Yes[14,50,51]
Alzheimer's disease	*PSEN1*, *PSEN2*, *APP*	*APOE*, *CR1*, *CLU*, *GSTO1*, *IDE*	No
Stroke	..	*PDE4D*, *ALOX5AP*, *SORBS1*, *FGG*, *FGA*, *LDL-PLA2*	No
Charcot–Marie–Tooth disease	*BSCL2*, *DNM2*, *EGR2*, *FGD4*, *FIG4*, *GARS*, *GDAP1*, *GJB1*, *HSPB1*, *HSPB8*, *KIF1B*, *LITAF*, *LMNA*, *MFN2*, *MPZ*, *MTMR2*, *NDRG1*, *NEFL*, *PMP22*, *PRPS1*, *PRX*, *RAB7A*, *SBF2*, *SH3TC2*, *YARS*	..	No

iPS=induced pluripotent stem. ALS=amyotrophic lateral sclerosis.

***Table 2*: Neurodevelopmental and neurodegenerative diseases potentially suitable for modelling with iPS cells**

established mainly in mouse and human ES cells, and have also been adapted to iPS cells (table 1).

Neurodegenerative and neurodevelopmental disorders suitable for modelling

The development of novel iPS cell models would be useful in many neurodegenerative and neurodevelopmental diseases (table 2). Neurodevelopmental disorders are particularly relevant for this type of modelling because iPS cells are, by definition, reprogrammed to an early, pluripotent state and, therefore, neural tissue generated from these cells could represent the early stages of disease. Indeed, the neuronal immaturity means that adult-onset diseases might be more difficult to model than earlier-onset diseases. The use of stressors (eg, excitotoxins, temperature shifts, growth supplement removal, etc) might be required to initiate specific phenotypes in adult-onset neurological diseases. These issues are discussed below for various diseases.

Neurodevelopmental disorders

Fragile X syndrome

Fragile X syndrome is inherited in an X-linked dominant way and is the most common inherited form of mental retardation.[52,53] It is caused by expansion of a trinucleotide sequence repeat of more than 200 CGG repeats in the 5′ UTR that silences *FMR1*.[54–56] The corresponding loss of the FMR1 protein leads to developmental changes within the cerebral cortex. Specifically, dendritic spines in this region of the brain are immaturely shaped.[57–60]

The expanded *FMR1* gene is expressed in the embryonic stages and is only silenced upon differentiation into adult cell lineages.[61] Models of fragile

X syndrome developed with human neural progenitor cells (NPCs) taken from fetal tissue have reproduced this lack of *FMR1* expression.[62] Reports of defective neurogenesis are conflicting,[62,63] but measureable changes have been seen in signal transduction genes in these human NPC models. Most of these changes were found within signalling pathways that might, when altered, have acute effects on the developing brain.[62]

iPS cells have been reprogrammed from fibroblasts from three different patients with fragile X syndrome.[45] The iPS cells continued to silence the expanded copy of *FMR1*, which would not be expected if the cells were pushed back to an embryonic state where the gene would normally be expressed.[61] The early expression of *FMR1* might, therefore, be better mimicked by ES cells. However, the FXS iPS cells still represent an exciting model to further study this disorder because they can be generated from living patients with a known phenotype and are capable of making neural tissues. Neurogenesis, cell death, and axonal sprouting were not assessed in neurons differentiated from these iPS cells.[45] *FMR1* is also a transcription regulator associated with around 4% of all fetal brain transcripts.[64–66] Differentiation of iPS cells into neurons and assessment for previously reported changes in transcript levels[67–73] might, therefore, reveal interesting results.

Rett's syndrome

Rett's syndrome is an X-linked disorder in the autism spectrum. Most cases are caused by spontaneous mutations in *MECP2*.[74] Most patients are female, as male fetuses or neonates with Rett's syndrome die, respectively, before or soon after birth. The severity of the syndrome in female patients is associated with the specific gene mutation and pattern of X-chromosome inactivation;[75] a study of *Mecp2*-deficient mice showed that the MECP2 protein has a crucial role in neuronal maturation.[76] This effect is thought to be due to abnormal expression profiles of genes involved in synaptogenesis.[75]

iPS cells have been generated from fibroblasts harvested from four patients with Rett's syndrome, from which selected clones underwent X-chromosome reactivation.[77] Upon differentiation, the cells underwent proper X-inactivation. However, no differences were seen in neural production or proliferation between Rett's syndrome iPS cells and those from controls. Neurons differentiated from the patients, however, had abnormal morphologies. Additionally, their electrophysiological phenotype was altered, in terms of lowered activity-dependent transient calcium concentrations in the cytosol and spontaneous postsynaptic currents. This finding suggests that neurons in these patients establish distorted neuronal networks.

Down's syndrome

Another developmental disorder for which iPS models might be useful is Down's syndrome. This disorder is caused by trisomy of chromosome 21. Thus, full recapitulation of the disease features in mouse models is difficult owing to mice not having a chromosome 21. ES cells have been generated from patients with Down's syndrome. In these cells, all three copies of chromosome 21 expressed transcripts.[78] This study also showed that during formation of the embryoid body, trisomy 21 substantially altered chromatin formation,[78] which is an essential process in cell differentiation and tissue development.

Human fetal NPCs have been used to develop models of Down's syndrome.[62,79] Studies show that other genes in addition to those on chromosome 21 are dysregulated[80] and that neurogenesis decreases over time in culture.[81] While these cells might be an excellent model for cortical development, senescence of human NPC at later passages in culture, when the decreased neurogenesis phenotype is most relevant to the actual cause of disease,[81] means that cells were not available for subsequent analysis. The generation of a Down's syndrome iPS model could enable continuous replay of cortical development, with use of reprogramming as the "reset" button, which would enable scientists to have an endless supply of diseased tissues to study. iPS cells were generated from patients with Down's syndrome,[14] but no differentiation studies were done. The creation of iPS cell lines to enable investigation of similar defects, such as trisomy in other chromosomes, would also be of interest.

Neurodegenerative disorders

Spinal muscular atrophy

Spinal muscular atrophy is the second most common autosomal recessive disorder and the most frequent genetic cause of infantile death. It is caused by the loss of function of SMN1.[82] For unknown reasons, the motor neurons in patients with spinal muscular atrophy are particularly susceptible to the loss of the SMN protein, which leads to cell death and muscle atrophy.[83] Spinal muscular atrophy has four subtypes that are classified by disease severity and age of onset, with type 1 being the most severe and type 4 being the least severe. Patients with type 1 disease account for around 60% of all new cases and typically do not survive past childhood. We have generated iPS cell lines from a patient with type 1 spinal muscular atrophy.[23] These cells were differentiated towards a motor-neuron lineage by use of previously described protocols[84,85] and expressed several markers specific to motor neurons. These iPS cells initially generated a similar number of motor neurons as their control cell counterparts, but over time cell body size was reduced and they underwent substantial degeneration.[23] Following on from these findings, multiple groups are screening for novel compounds that might increase SMN protein expression with the aim of improving motor neuron survival in these patients.

Huntington's disease

Huntington's disease is an autosomal dominant neurodegenerative disorder caused by expanded CAG repeats in exon 1 of *Huntingtin (HTT)*.[86] People with

fewer than 35 repeats do not develop the disease, those with 36–40 repeats are at risk, and those with more than 40 repeats develop Huntington's disease. Disease severity increases and the age of onset is reduced in direct correlation with an increasing number of repeats; more than 60 CAG repeats leads to early-onset Huntington's disease. The expanded repeat region causes a gain of function in the huntingtin protein, which then forms aggregates within the nucleus of certain neuronal cells. The huntingtin protein is ubiquitously expressed but the medium spiny neurons of the striatum and the cortex are primarily affected. The reason for this selectivity is unknown.

Many tissue culture models for Huntington's disease have been generated by a wide range of techniques. Non-neural human cell types, such as fibroblasts and lymphoblasts, show some defects in the ubiquitin-proteasome system[87] or A2A receptor function,[88] although how these relate to brain degeneration is unclear. Rodent models use CNS tissues, such as immortalised or primary neurons that overexpress mutant huntingtin. These models can recapitulate many of the phenotypes seen in patients with Huntington's Disease.[21,89,90] Mouse ES cells have also been generated from Huntington's disease model in knock-in mice (expressing 150 CAG repeats)[91] and human ES cells have been obtained from affected embryos diagnosed by genetic testing during selection for in vitro fertilisation (expressing 37 and 51 CAG repeats).[92] Neurons generated from the mouse ES cell model of Huntington's disease differentiated more quickly than wild-type mouse ES cells[91] and, although no overt phenotype was demonstrated, genomic CAG instability in the differentiated cells was noted. Such genomic CAG instability is also seen in somatic tissue samples from patients.[93] In a different study, human ES cells were generated from four affected embryos that expressed 40, 45, 46, and 48 CAG repeats, respectively.[94] These ES cells were clearly pluripotent and could generate neurons upon differentiation, but no phenotypical analysis was done.

Park and colleagues[14] generated iPS cells from a patient with Huntington's disease displaying 72 CAG repeats. These cells have been used to generate striatal neurons susceptible to cellular damage characteristic of the disease, such as mutant huntingtin aggregation and decreasing concentrations of glutamate transporters and BDNF.[48] Because disease onset generally occurs in later life, an overt cell death phenotype was, perhaps unsurprisingly, not seen in these cells. Removal of growth factors, however, led to increased caspase activity in the Huntington's disease cell line compared with that in control cells. This finding is indicative of apoptosis. In the future, modelling of other phenotypes currently only seen at autopsy might become possible, such as huntingtin aggregation, astrogliosis, and downregulation of messenger RNA encoding BDNF and glutamate receptors or transporters. Additionally, electrophysiological changes seen in animal models (eg, dysfunctional burst activity,[95] greatly reduced spontaneous excitatory postsynaptic currents,[96] and increased input resistances[97]) might also be detected as iPS cell phenotypes in Huntington's disease.

Parkinson's disease

Parkinson's disease involves degeneration of the neurons within the substantia nigra that produce dopamine, followed by other degenerative changes throughout the brain. Disease onset occurs typically in later adult life but, like in Huntington's disease, there are also early-onset forms. Many genes have been directly associated with Parkinson's disease (*PARK2, SNCA, UCHL1, LRRK2, PARK7, PINK1, GBA*, and *SNCAIP*),[98] although more than 90% of Parkinson's disease cases seem to be sporadic.

ES cells can be differentiated into the dopamine neurons affected in Parkinson's disease.[99] A mouse ES cell model of Parkinson's disease has been generated by overexpression of mouse Nr4a2 (previously Nurr1) and mutant α-synuclein.[100] When differentiated into dopaminergic neurons, these cells are more susceptible to oxidative stress and proteasome and mitochondrial inhibition than control cells, resulting in the death of the cells over the course of about 1 month in culture. In a model in which dopaminergic neurons were generated from human ES cells, more cells died after overexpression of α-synuclein than in the control group.[101] None of these lines, however, had the genetic background of a patient with Parkinson's disease. Preimplantation genetic testing of embryos during selection for in-vitro fertilisation is only available for cases with a single mutation. Thus, acquisition of sporadic Parkinson's disease ES cells by this method would be very difficult because in most cases the genetic factors are unknown.

Generation of iPS cells from patients with Parkinson's disease has been described in three reports.[14,50,51] No phenotype of any sort was initially reported for the Parkinson's disease iPS cells.[34,50] Some evidence has shown, however, that after iPS cells with a mutation in *PINK1* were differentiated into dopaminergic neurons, that recruitment of PARK2 to the mitochondria was impaired upon mitochondrial depolarisation, and the number of mitochondria were increased.[51] Interestingly, these phenotypes were all reversed after overexpression of wild-type *PINK1*. These lines will be extremely useful in further modelling the genesis of Parkinson's disease and also for therapeutic screening.

Amyotrophic lateral sclerosis (ALS)

ALS is an adult-onset disease with some genetic basis, although most cases are sporadic. Many genes have been implicated, such as *SOD1, DPP6, ITPR2*, and *TARDBP* (also known as *TDP43*). ALS generally presents between the fourth and sixth decades of life and is caused by the death of upper and lower motor neurons, which leads to paralysis and subsequent atrophy of the muscles.

Tissue culture models of ALS have been created by overexpression of mutant *SOD1*. Neuroblastoma cells overexpressing this gene do not show any decrease in viability after differentiation, but do have increased susceptibility to oxidative stress.[102] Motor neurons differentiated from ES cells have decreased viability after coculture with mouse astrocytes overexpressing mutant *SOD1*.[103,104] These models have no doubt contributed greatly to the current knowledge of disease mechanisms. An iPS cell model that produces human motor neurons with a genetic background that truly reflects the multigenic nature of this disease (as *SOD1* mutations are only seen in a small percentage of patients) might, however, generate a greater understanding of the mechanisms underlying this disorder.

In 2008, iPS cells were generated from a skin sample taken from an elderly patient with familial ALS displaying a mutation in *SOD1*.[24] Motor neurons with the proper immunological markers HB9 and Islet 1 were generated from these cells. The number of motor neurons generated from the ALS iPS and control cell lines, however, were not reported. Such information could be of importance in future studies of defects in motor neuron production and survival. Differentiation studies are now needed to establish disease-specific differences between motor neurons from patients with ALS and control cell lines.

Issues in implementation of modelling with iPS cells

Modelling of neurodegenerative disease with iPS cells will have to overcome many hurdles owing to the limited experience with this technology so far, but also possesses great promise. We discuss below some of these issues and how model development might move forward.

Rapid development of induced pluripotent stem-cell technology

The first technical challenge is the generation of iPS cells. Initially, researchers used lentiviruses to transduce pluripotentcy genes into somatic cells. With this technique, however, integration into the genome can be random. This might lead to disruption of endogenous genes, creating the potential for tumourigenesis or other changes in cell proliferation or differentiation in culture. Therefore, other methods of delivering pluripotency are being assessed. The first revision of this method involved the combination of all the genes onto an expression cassette to limit the number of integration sites.[105,106] Another method used was the Cre-Lox system, which enables the removal of pluripotent genes from the genome after reprogramming.[49,107] Briefly described, a gene is engineered to have specific flanking sequences (called loxP sites) before integration into the genome. Then, at a desired time a Cre protein (a DNA recombinase) can be expressed that cuts the DNA at the loxP sites, thereby removing the gene of interest. This approach, however, still leaves a small region of vector sequence inserted in the genome. To avoid integration all together, non-integrating viruses,[108,109] excisable piggyBac DNA transposons,[110] episomal vectors,[111] and repeated transfections[112] have been used. Various proteins, modified messenger RNA,[113] and compounds are being investigated for their ability to reprogramme the cells, either alone[114–116] or in conjunction with viruses encoding pluripotency genes.[117] This move from classic reprogramming by gene transfer to new approaches that use proteins and compounds might trigger a new wave of discoveries in the iPS cells field.

Can iPS cells generate every neuronal lineage?

Differentiation protocols do not exist for generation of all cell types in the CNS, and many are extremely time consuming and might or might not generate a good amount of the desired cell type. Efficiency needs to be improved if these protocols are to be used to generate models involving iPS cells for high-throughput drug screening. The CNS comprises many cell types and they might act differently when taken out of their endogenous settings, and therefore might not survive. Furthermore, multiple protocols exist to obtain iPS cells, neural induction,[23,118–120] and differentiation (figure 2), which creates inconsistency between research groups. For instance, the starting points of growing the pluripotent cells can differ dependent upon the growth substrate, such as matrigel versus MEFs or SNLs (an immortalised MEF cell line that stably expresses leukaemia inibitory factor [LIF] and neomycin resistance) and the formulation of the cell media. The method of neural induction also varies tremendously between laboratories (embryoid bodies, adherent monolayers,[118] rosette formation,[119] stromal feeder layers,[119,120] EZ spheres,[23] etc). With so much variation, replication of results can be very difficult. Therefore, any phenotype assessed in models that use iPS cells would need to be robust enough for replication by diverse methods of differentiation.

The major hurdle to overcome before patient-derived iPS cells can be used for modelling of neurological diseases is the identification of phenotypes to assess late-onset disorders. This identification might be particularly, because reprogramming reverses ageing effects seen in the primary tissues.[121] Therefore, to potentially uncover a phenotype, the iPS cells need to be artificially and rapidly aged after full differentiation. The phenotype does not need to be overt cell death, but may be a more-subtle but robust alteration in the cells' physiology. Several methods exist to induce artificial ageing effects in cells, but the most frequently used is to continually stress the cultures by withdrawal of growth factors or addition of neurotoxins to the culture media. Use of these methods might in turn provide clues about the causal mechanisms underlying diseases.

Whether to look at specific neuronal populations susceptible to disease in isolation or in a mixed culture

might depend on the disease being studied. For instance, would iPS cells taken from patients with ALS be better analysed in a single culture? In some ways, to study iPS cell-derived motor neurons in isolation would be extremely beneficial because contamination of products by unaffected cells would not be an issue, and cell-autonomous mechanisms of disease could be pinpointed. However, astrocytes are also implicated in disease progression in patients with ALS[122,123] and, therefore, to study these cells in a mixed culture might also be useful, even though astrocyte survival might not be affected by the disease. The presence of muscle cells in the cultures as targets for the motor neuron might also be useful, as active motor neurons might be more susceptible to death than those that are not firing. The various approaches need to be carefully assessed.

Non-genetic causes of disease

Many cases of neurodegenerative diseases, such as Parkinson's disease and ALS, seem to be sporadic. Most patients, however, may have an unidentified genetic component that is coupled with environmental factors. In theory generation of iPS cells leads to complete reprogramming, but these cells might retain some epigenetic memory from their primary source tissue.[124] The role of the disease-triggering environmental factors on disease might not be reflected in models involving reprogrammed cells. Subsequently, the iPS cells might no longer reflect pathogenesis. Such an outcome was seen in a cancer model reported by Ron-Bigger and colleagues.[125] Careful study of genetic versus environmental contributions to a disease phenotype by use of iPS cell models could increase understanding of the underlying mechanisms.

Future directions for modelling of neurodegeneration

Isogenic cell lines

One notable potential of modelling with iPS cells is the development of isogenic cell lines. Currently, many cell models rely on the acquisition of a sample from an unaffected family member to provide a control of genetic variance within the population.[23] New technologies, such as those that use zinc finger nucleases can target a double-stranded break in a specific sequence of interest and enable homologous recombination at that exact location.[126] Thus a control cell line can be generated simply by fixing the defective gene in the iPS cells generated from the patient. Although a fix could be done with traditional homologous recombination techniques, zinc finger nucleases provide greater specificity[127] and efficiency.[128] This method ensures that the genetic backgrounds of the study cells and the control cell lines are exactly matched except for the gene that underlies the disease.

Isogenic generation of iPS cell lines used for disease modelling would be akin to the use of inbred mouse strains for animal models, and might lead to the creation of standard protocols. This technique would, however, apply only to diseases with known genetic causes (table 2) and would preclude modelling of many neurodegenerative diseases with sporadic forms. Diseases characterised by single-gene defects would be the most suited to this method of modelling, owing to the simple target for zinc finger nucleases. For instance, extended CAG repeats could be removed from *HTT* and replaced with a non-pathogenic number of repeats to provide controls in models of Huntington's disease. Diseases with multiple genetic causes might, however, also be suitable. For example, in cells taken from a patient with ALS carrying a known *SOD1* mutation, this mutation could be repaired and the cell line used as a control. Some mutations might, however, be seen in only a small subgroup of patients and, therefore, findings would need to be validated with other models of other known mutations.

Personalised treatment

Another potential use of iPS cells is personalisation of treatment. If scientists could overcome the hindrances to reprogramming, it would be feasible to generate iPS cells from every patient. These could be used to screen for drugs in each individual patient.[129] This approach is conceptually similar to the culture of bacterial infections to find the most effective antibiotic regimen. In the future this type of patient-specific drug screening might be feasible and would be particularly helpful in disorders where patients frequently have to try multiple drugs before finding a regimen that works.

If patient-specific iPS cells were available, screens of drugs could be performed to examine exactly which drug would work best for that patient.[129] Another use for these iPS cell models would be to study genes that increased susceptibility to disease, rather than being causal. For instance, some mutations in *APOE* alleles increase susceptibility to Alzheimer's disease.[130] Therefore, iPS cells could potentially be used to assess whether a particular person with increased susceptibility would or would not actually develop disease. While individualised modelling with iPS cells remains a propect for the future and, of course, would be expensive, it might revolutionise modern medicine.

Transdifferentiation techniques

Vierbuchen and co-workers[131] reported that fibroblasts can be directly reprogrammed into neurons without the need for reverting to the pluripotent state. Thus, reprogramming could be much more rapid than with previous differentiation protocols. The studies, however, were performed in mice and have not yet been fully replicated in human tissues. Furthermore, neurons generated by direct reprogramming would in theory have very little potential for expansion because neuronal cells are non-mitotic. Thus, this method of reprogramming

might not be useful for models of neurodegenerative diseases, which require large numbers of neurons.[132] Although these original studies were done on embryonic and postnatal tissues, it has recently been proven that adult fibroblasts can be transdifferentiated into blood progenitors, proving that adult tissues can also be transdifferentiated.[133] Therefore, it will be interesting to validate that older tissues can also be used to generate neurons.

Genomic stability

A current challenge for the field of iPS cell modelling is the understanding of the mechanisms of genomic stability in these cells. Several recent publications have shown both gross karyotypic abnormalities,[134–136] and more subtle mutations[137–140] in stem cells. Gross karyotypic abnormalities, such as chromosomal aneuploidy, can cause increased tumourigenicity and altered differentiation capabilities. Additionally, although some subtle mutations may exist within protein coding frames naturally in somatic tissues throughout the general population, it has been shown that even more arise during reprogramming. Many of these coding mutations are found in oncogenic genes, which may lead to tumourigenicity. Unfortunately, these karyotypic abnormalities and mutations seem to be inevitable when cells are propagated in culture. Therefore, as iPS cells are used for modelling of neurodegenerative diseases, monitoring of genomic stability must be a standard procedure both at creation of a line and through its propagation.

Conclusions

In-vitro iPS cell models of neurodegenerative and neurodevelopmental disorders have both benefits and drawbacks. All diseases might not be amenable to modelling with iPS cells, but the ability to derive these cells from individual patients strengthens the potential and relevance of disease modelling by circumvention of many of the issues seen with current animal models and immortalised or tumorigenic cell lines. Patient-derived cells open new avenues of research for sporadic disorders or those with unknown genetic backgrounds, and offer an ideal platform for high-throughput drug screening.[141] However, differences in gene expression, epigenetic states, and differentiation between established human ES cells and new iPS cells need to be resolved.[23–45] Furthermore, although many cell types have been differentiated in vitro from human ES cells, most have been generated with low efficiency and some cell types have yet to be generated. Unfortunately, iPS cells will not be useful if cell types vulnerable to disease cannot be differentiated in high enough quantities.

What constitutes an appropriate control cell line remains an issue. For instance, consanguineous controls might carry modifier genes that could change the outcomes of the study. Additionally, control cell lines are mainly generated from age-matched family members, but the individuals are generally not followed up to find out whether they develop the same or another neurological disease. Ultimately, multiple control and disease lines (perhaps ten or more of each) will be needed to confirm disease-specific phenotypes.

For all the challenges that lie ahead for this new and rapidly evolving specialty, one thing is certain. We now have access to novel models in which human neurological diseases can be replayed over and over again in the culture dish. We predict that this new technology will complement the cell and whole-animal models currently available and will lead to new insights into neurological illness.

Search strategy and selection criteria

Articles published from January, 1981, to February, 2011, were identified through searches of PubMed with the search terms "iPS cells", "ES cells", "pluripotent", "iPS OR ES model", "neurodegeneration", "neurodegenerative disease", "neurodegeneration model", "reprogramming", "Spinal Muscular Atrophy", "Down Syndrome", "Huntington Disease", "Parkinson Disease", "Fragile X", and "Amyotrophic Lateral Sclerosis". Only papers published in English were reviewed.

Contributors

Both authors contributed the ideas for and wrote the Review. VBM did the literature searches and prepared the figures and tables.

Conflicts of interest

We have no conflicts of interest.

Acknowledgments

We thank Soshana Svendsen for useful comments and proofreading.

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