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Review

Remodeling Neurodegeneration: Somatic Cell Reprogramming-Based Models of Adult Neurological Disorders

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Epigenetic reprogramming of adult human somatic cells to alternative fates, such as the conversion of human skin fibroblasts to induced pluripotency stem cells (iPSC), has enabled the generation of novel cellular models of CNS disorders. Cell reprogramming models appear particularly promising in the context of human neurological disorders of aging such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), for which animal models may not recapitulate key aspects of disease pathology. In addition, recent developments in reprogramming technology have allowed for more selective cell fate interconversion events, as from skin fibroblasts directly to diverse induced neuron (iN) subtypes. Challenges to human reprogramming-based cell models of disease are the heterogeneity of the human population and the extended temporal course of these disorders. A major goal is the accurate modeling of common nonfamilial "sporadic" forms of brain disorders.

Introduction

The architectural complexity and cellular diversity of the mammalian brain represent major challenges to the pursuit of etiological factors that underlie human degenerative brain disorders. A further impediment particular to the analysis of degenerative brain diseases is their protracted time course. And although animal models have greatly informed current views on these disorders, they have often failed to recapitulate key aspects of the diseases. Thus, reductionist in vitro approaches using human cells, such as the analysis of patient-derived neurons generated using iPSC, have been met with particular excitement (Abeliovich and Doege, 2009; Takahashi and Yamanaka, 2006; Yamanaka, 2007). More recent advances offer a variety of additional tools, such as for the genetic correction of disease-associated mutations in patient-derived cultures. Even with such advances, cell-based approaches to study human neurodegenerative diseases are limited by the inherent genetic diversity of the human population, as well as technical variation among accessible human tissue samples. Recent studies using human reprogramming-based cell models of neuronal disorders have brought a number of mechanistic topics to the fore, including the significance of non-neuronal or non-cell-autonomous factors in disease, the relevance of epigenetic mechanisms, and the potential of cell-based drug discovery approaches.

Cell-Fate Plasticity and iPSC Reprogramming

Pioneering studies in Shinya Yamanaka's laboratory established that a cocktail of four pluripotency factors —OCT4, SOX2, KLF4, and c-MYC (OSKM), encoded in viral expression vectors—could effectively reprogram skin fibroblasts to a pluripotent cell fate within a few weeks (Takahashi and Yamanaka, 2006; Yamanaka, 2007; Figure 1). Although iPSC may not precisely replicate the epigenetic state of embryonic stem cells (ESC) (Kim et al., 2010), they functionally recapitulate key ESC attributes, such as the seemingly unlimited ability to self-renew, as well as the capacity to differentiate into a broad spectrum of somatic cell fates. An early concern of the iPSC methodology was that random insertion of the lentivirus vectors into the host genome might adversely impact cells, leading to untoward phenotypic changes such as tumor transformation. Alternative methods for gene transduction, including the use of nonintegrating viral vectors such as Sendai virus (Ban et al., 2011), episomal vectors (Okita et al., 2011), protein transduction (Kim et al., 2009), or transfection of modified mRNA transcripts (Warren et al., 2010), have now been developed to mitigate such concerns. These technologies are relevant both in the context of any future clinical applications of iPSC as transplantable replacement cell therapies, and as reductionist in vitro model systems in which to pursue and validate therapeutic approaches for CNS disorders. The latter application has advanced significantly since the initial description of iPSC by the Yamanaka group (Takahashi et al., 2007).

iPSC can be efficiently differentiated into a variety of neuronal or nonneuronal fates, using a growing toolbox of differentiation protocols. These protocols often take advantage of existing knowledge about in vivo pathways that drive mammalian CNS embryonic development. For example, Studer and colleagues (Fasano et al., 2010; Kriks et al., 2011) described the efficient production of multipotent neural stem cells with a ventral floor plate phenotype—as defined by a transcription factor expression profile and competence in the generation of several ventral floor-plate derived cell fates. The protocol is based on concurrent inhibition of two parallel SMAD/transforming growth factor-β (TGFβ) superfamily signaling pathways—mediated by bone morphogenic proteins (BMP) and Activin/Nodal/TGFβ (Muñoz-Sanjuán and Brivanlou, 2002). As these signaling

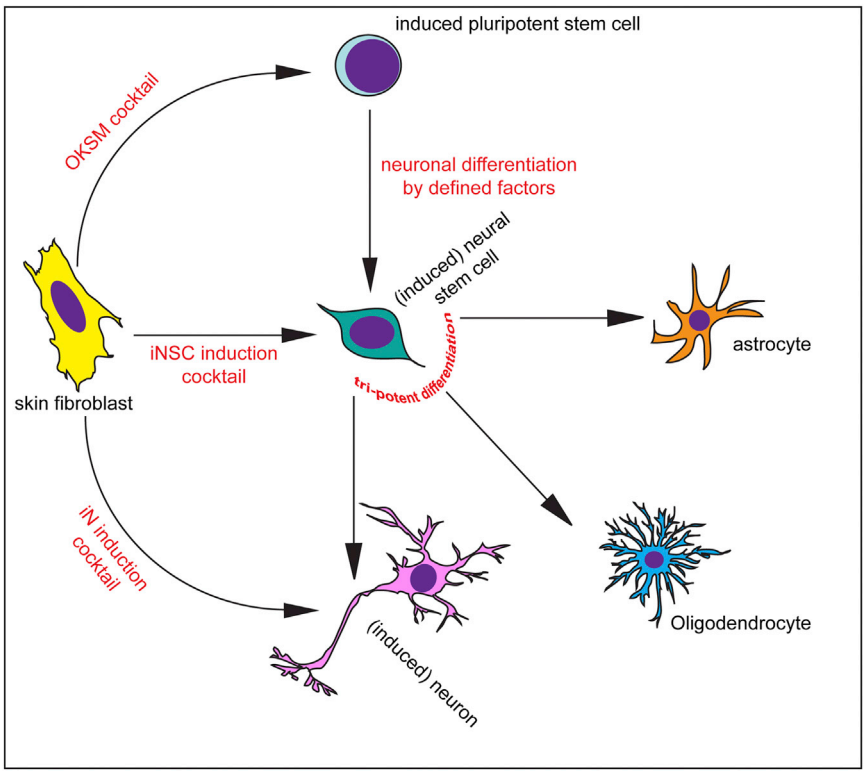

Figure 1. Schematic of Reprogramming-Based Approaches for the Generation of Human Neuron Models of Disease

These approaches include the generation of iPSC that are then differentiated to mature neurons, directed conversion to induced neurons or directed conversion to dividing neural progenitors, that can give rise to mature CNS cell types.

pathways typically induce nonneuronal fates such as epidermis or mesoderm during CNS development, their concurrent inhibition promotes a "default" neural progenitor fate, which resembles tripotent neural stem cells. Subsequent differentiation of these neuronal stem cells toward selected mature neuronal types can be achieved by inducing yet other signaling pathways (Chambers et al., 2009, 2012; Kriks et al., 2011). For instance, a mature midbrain dopaminergic neuron fate can be instructed by Wnt, Sonic hedgehog (SHH), and FGF8 signaling pathway induction, using chemical compounds or endogenous ligands. Similar approaches have been described for the efficient production of other neuronal fates, such as glutamatergic telencephalic forebrain neurons (Kirkeby et al., 2012), limb-innervating motor neurons (Amoroso et al., 2013), and neural crest derivatives (Chambers et al., 2009; Greber et al., 2011; Lee et al., 2007; Table 1).

Some limitations to iPSC technology have emerged. Although iPSC appear phenotypically stable through many cell divisions, consistent with the self-renewal properties of stem cells, careful inspection of the genomic DNA from iPSC has revealed a propensity toward the accumulation of genomic aberrations with extended culturing (as well as the selection of any existing mutations in skin fibroblasts that may confer a clonal growth advantage) (Gore et al., 2011; Hussein et al., 2011; Laurent et al., 2011). An additional issue with human iPSC technology has been the lack of a standardized and practical method to authenticate pluripotency (in contrast to rodent iPSC, which can be authenticated for pluripotency by germline transmission). A common approach has been the generation of teratomas—tumors which harbor a broad variety of cell types—upon transplantation of iPSC into rodent tissue in vivo. However, this method is cumbersome, particularly for studies that necessitate the generation of large cohorts of independent iPSC clones, and can be misleading—even aneuploidy cultures are competent at the formation of teratomas. An alternative approach to assess pluripotency potential is through gene expression and epigenetic marker analyses, which appear predictive (Bock et al., 2011; Stadtfeld et al., 2010, 2012). An added layer of complexity is that individual iPSC clones—even within the same reprogramming culture dish—may show significant phenotypic variability, due either to the acquisition of new genomic mutations as above, or to the epigenetic heterogeneity, which remains poorly understood (Gore et al., 2011; Hussein et al., 2011; Laurent et al., 2011).

Directed Reprogramming: A Shortcut

The success of iPSC reprogramming has informed the pursuit of other forms of somatic cell-fate conversion, such as directed conversion from skin fibroblasts to forebrain neurons, termed induced neurons (iNs) (Ambasudhan et al., 2011; Caiazzo et al., 2011; Chatrchyan et al., 2011; Pang et al., 2011; Pfisterer et al., 2011; Qiang et al., 2011; Vierbuchen et al., 2010; Yoo et al., 2011). Directed conversion methods have taken essentially the same conceptual strategy as with iPSC generation but are based on the transduction of an empirically determined "cocktail" of candidate neurogenic factors, rather than pluripotency factors. A factor common to most of the directed conversion protocols is ASCL1 (also termed MASH1), a basic helix-loop-helix (bHLH) proneural gene that is required for the generation of neural progenitors during embryogenesis and in the adult (Casarosa et al., 1999; Nieto et al., 2001; Parras et al., 2002; Ross et al., 2003), as well as for subsequent specification of some mature neuronal subtypes (Lo et al., 2002). Additional conversion factors may primarily impact on the neuronal subtype generated, akin to the role of "terminal selector" factors during in vivo development (Hobert, 2008). For instance, generation of rodent iN cells with a forebrain glutamatergic phenotype was initially described using an ASCL1/MYT1L/BRN2 cocktail (Vierbuchen et al., 2010). Such iN conversions in rodent and human (hiN) cultures appears qualitatively comparable (Pang et al., 2011; Qiang et al., 2011;

Table 1. A Summary of Human iPSC Differentiation Protocols toward Mature Neuronal Fates

Neuronal Subtypes	Key Components for Differentiation or Reprogramming	Efficiency	Duration	Reference
Glutamatergic neurons	hiPSC-derived EB formation followed by dissociated cultures in the absence of exogenous growth factors and serum	~85% from hiPSC to NPs; >60% from NP to neurons	~25 days to NPs with additional 5 weeks to the neuronal phenotype	Zeng et al., 2010
GABAergic neurons	Low levels of SHH and Wnts	~87%	5–6 weeks	Liu and Zhang, 2011
Cholinergic neurons	High level of SHH and low level of Wnts[a], BMP9, and NGF promote the yield	14%–38%	N/A	Liu and Zhang, 2011; Schnitzler et al., 2010
Dopaminergic neurons	Noggin and SB431542 followed by SHH and FGF8, then exposure to BDNF, AA, GDNF, TGFβ3, and cAMP	82% neural stem/ precursor induction	~19 days	Kriks et al., 2011
Motoneurons	Noggin and SB431542 followed by BDNF, AA, SHH, and RA	82% neural stem/ precursor induction	~19 days	Chambers et al., 2009
Serotoninergic neurons	Matrigel and noggin without EB formation or additional factors.	~80%	14 days	Shimada et al., 2012
NCSCs/Peripheral neurons	GSK-3β inhibitor and SB431542 /BDNF, GDNF, NT3, AA, cAMP	>90% from iPSCs to NCSCs; 70%–85% from NCSCs to neurons	~15 days from hiPSC to NCSCs; 12–14 days from NCSCs to peripheral neurons	Greber et al., 2011; Menendez et al., 2013

Abbreviations: EB, embryonic body; NPs, neural progenitors; SHH, sonic hedgehog; Wnt, wingless-int; BMP, bone morphogenetic protein; NGF, nerve growth factor; FGF8, fibroblast growth factor 8; BDNF, brain-derived neurotrophic factor; GDNF, glial-derived neurotrophic factor; TGFβ, transforming growth factor β; cAMP, cyclic AMP; AA, ascorbic acid; RA, retinoic acid, GSK-3β, glycogen synthase kinase-3 β; NCSCs, neural crest stem cell.
[a]The yield of cholinergic neurons is still very limited.

Vierbuchen et al., 2010; Yoo et al., 2011), although the conversion efficiency appears generally lower in human cultures. Directed reprogramming has been described toward spinal motor neurons (iMN) (Son et al., 2011) and midbrain dopaminergic neuron (iDA) fates as well (Caiazzo et al., 2011; Kim et al., 2011b; Pfisterer et al., 2011), using alternative neurogenic regulatory cocktails (Table 2). At this time, directed conversion does not offer as broad a selection of potential differentiated cell fates as with iPSC differentiation protocols.

Directed conversion is operationally defined as a process that does not follow a known mammalian neuronal developmental pathway, nor the circuitous course of iPSC-derived neuron generation through a pluripotent intermediate. Unlike iPSC generation, which is inefficient to the point that only a handful of individual iPSC clones are typically obtained in a transduced culture (~0.1% of transduced cells are reprogrammed to pluripotency), a feature of the directed conversion methods is the relatively higher efficiency (~10% of cells may be converted) (Qiang et al., 2011; Vierbuchen et al., 2010). Remaining cells in iN cell cultures appear fibroblastic, and may be purified away using fluorescent-activated cell sorting with neuronal markers such as the neural cell adhesion molecule (NCAM) (Qiang et al., 2011). The higher efficiency of conversion described with the iN approach obviates the need for clonal expansion of individual reprogramming events; such cloning, as in the context of iPSC generation, may theoretically bias subsequent phenotypic analyses. iNs are postmitotic and display typical neuronal morphological features and markers, as well as active membrane properties of neurons (Pang et al., 2011; Qiang et al., 2011; Vierbuchen et al., 2010; Yoo et al., 2011). The iN conversion method appears robust in that nonfibroblastic cell types, such as hepatocytes, also appear amenable to hiN conversion (Marro et al., 2011). Directed in vitro conversion offers some potential advantages over iPSC reprogramming in the context of disease modeling. The relative simplicity of the hiN methods—the process typically takes approximately 3–4 weeks and can be miniaturized to a multi-well format—may enable the analysis of large patient cohorts without a tremendous investment. However, as with iPSC reprogramming, the process is highly dependent on the fidelity of the source cells, such as skin fibroblasts.

Initial studies with neurons derived by directed conversion, in rodent or human cultures, suggested functional immaturity (Pang et al., 2011; Qiang et al., 2011; Vierbuchen et al., 2010;

Table 2. A Summary of Directed Reprogramming Methods for the Generation of Human CNS Cell Phenotypes

Original Cells	Target Cells	Key Components for Differentiation or Reprogramming	Efficiency	Duration	Reference
Human fibroblasts	Glutamatergic/ GABAergic neurons	Ascl1, Brn2, Myt1l, Oligo2, Zic1	9%	2–3 weeks	Qiang et al., 2011
		miR-9/9*, miR-124, Ascl1, Myt1l, NeuroD2	N/A	6 weeks	Yoo et al., 2011
		Ascl1, Brn2, Myt1l, NeuroD1	4%	2–5 weeks	Pang et al., 2011
		Ascl1, Brn2, Myt1l	4%	2 weeks	Pfisterer et al., 2011
		Brn2, Myt1l, miR-124	4%–11%	2–3 weeks	Ambasudhan et al., 2011
	Dopaminergic neurons	Ascl1, LmX1a, Nurr1	3%–6%	2–3 weeks	Caiazzo et al., 2011
		Ascl1, Brn2, Myt1l, Lmx1a, FoxA2	5%–10%	3–4 weeks	Pfisterer et al., 2011
	Motoneurons	Ascl1, Brn2, Myt1l, NeuroD1, Lhx3, Hb9, Isl1, Ngn2	0.05%	4–5 weeks	Chatrchyan et al., 2011
	Neural stem cells	Sox2	N/A	1–2 weeks	Ring et al., 2012

Yoo et al., 2011), in that "spontaneous" synaptic activity—suggestive of functional synapses—was not readily apparent. Addition of exogenous astrocytes, which are known to supply essential factors for synaptogenesis in other primary neuron culture models (Eroglu and Barres, 2010; Ullian et al., 2004), "rescued" this phenotype. More recently, the issue of functional maturity has also been addressed by altering the composition of the neurogenic factor cocktail. For instance, transduction of a cocktail of factors that includes miR-124—a highly expressed neuronal microRNA that modulates expression of antineuronal gene regulatory factors, such as REST, during CNS development (Ambasudhan et al., 2011) —appeared effective in generating mature neurons with evidence of spontaneous synaptic activity. In a related approach, repression of poly-pyrimidine-tract binding protein (PTB), which is thought to normally oppose the action of miR-124, appeared sufficient to convert fibroblasts to a neuronal phenotype (Xue et al., 2013). Circumventing the need for ASCL1 or other additional exogenous regulatory factors significantly simplifies the conversion process.

Extrinsic cues also play a major regulatory role in the neuronal fate conversion process. Withdrawal of serum, and inclusion of neurotrophic factors, is a common feature in the directed reprogramming protocols. Small molecule antagonists of glycogen synthase kinase-3β (GSK-3β) and SMAD signaling—signaling pathways implicated in CNS neurogenesis in vivo—have been reported to significantly improve the efficiency of reprogramming (Ladewig et al., 2012). Addition of exogenous primary astrocytes, which likely provide essential factors for synaptic maturation (Ullian et al., 2004), effectively promote synaptic activity in the iN cultures (Pang et al., 2011; Qiang et al., 2011; Vierbuchen et al., 2010; Yoo et al., 2011). A reduction in oxygen tension to physiological levels (Davila et al., 2013) may also promote the generation of mature neurons in vitro by directed conversion.

A particularly intriguing and potentially clinically relevant application is the directed conversion of nonneuronal cells to neurons in the adult CNS environment in vivo. In the adult mammalian CNS, switching cell fates has appeared to be particularly restricted, even from one neuronal type to another, although such switching has been described during late development (Rouaux and Arlotta, 2013). Genetic studies in the nematode *C. elegans* have achieved the efficient in vivo directed conversion of mature germ cells directly into neurons, by elimination of chromatin regulatory factors (Tursun et al., 2011); it is unclear whether such a strategy would promote directed fate interconversion in the adult mammalian CNS. Another drawback to the directed generation of mature, postmitotic hiNs, is that these cells cannot be further propagated. Thus for applications requiring large cell numbers, the method necessitates an initial amplification of the precursor fibroblasts.

An alternative, intermediate approach toward the generation of new neurons is the directed conversion of skin fibroblasts to a tripotent neural stem cell fate (Figure 1), termed induced neural stem cells (iNSC). iNSC remain capable of cell division and differentiation into a variety of CNS cell types (Han et al., 2012; Kim et al., 2011a; Lujan et al., 2012; Ring et al., 2012; Thier et al., 2012), including neurons, astrocytes, and oligodendrocytes. Interestingly, the same set of OSKM pluripotency factors, as described in the original iPSC protocol of the Yamanaka group, appears sufficient for directed conversion to iNSC, depending on the presence of iNSC permissive medium. Additional studies indicate that transient, rather than sustained, OCT4 expression is optimal for iNSC conversion (in contrast to iPSC generation) (Thier et al., 2012), and furthermore that SOX2 alone appears sufficient for the iNSC reprogramming process in some contexts (Ring et al., 2012). The absence of expression of pluripotency markers during iNSC reprogramming argues that the process is truly "directed," rather than simply an accelerated form of the iPSC-mediated generation of neurons through a pluripotent intermediate. Directed conversion to iNSC may prove particularly applicable for CNS disease modeling, insofar as it may marry the scalability of iPSC methods with the relative simplicity of directed reprogramming.

Cell Reprogramming-Based Models of Adult Neurological Disorders

A key promise of reprogramming-derived patient neurons for the study of neurological disease is to achieve truly "personalized" medicine, as for identifying therapeutics that would be most effective in a given patient (Figure 2). However, before such a goal can be reached, informative disease-associated cell models need to be validated. There is a growing list of neurological disorders that have been pursued using reprogramming technologies, primarily based on iPSC-derived patient neuron cultures (Table 3). Although this review does not detail all studies,

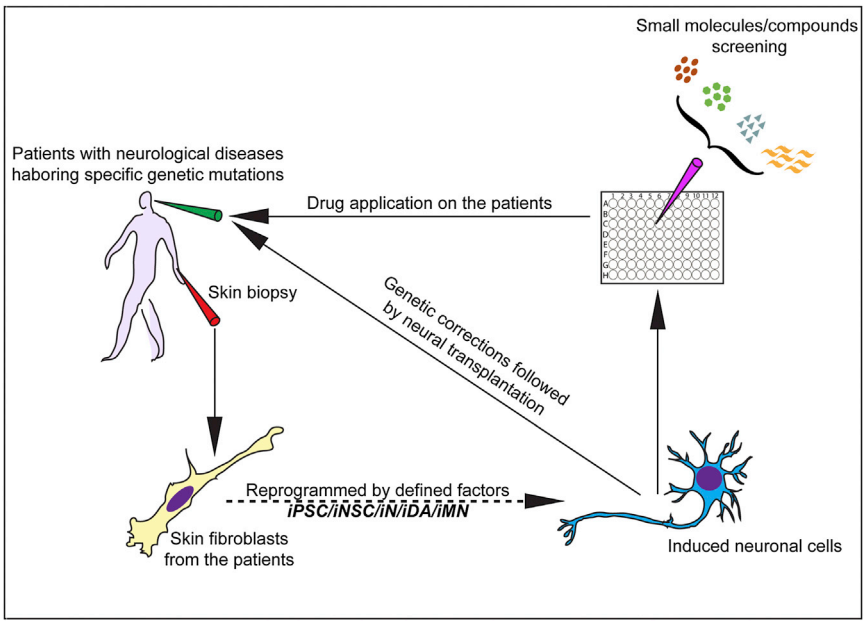

Figure 2. Therapeutic Strategies and Somatic Cell Reprogramming Technologies
"Personalized medicine" approaches include the generation of autologous "replacement" cells, as well as the identification of therapeutics that are particularly effective for a given individual.

several themes are considered. The use of human skin fibroblasts from older individuals presents some technical hurdles irrespective of the disease focus or the methodology. iPSC and iN technologies have been successfully applied to human cultures from older individuals, but efficiencies are typically lower than in cultures from rodents, and validation more complex. The basis for the lower reprogramming efficiency seen with cells from older individuals is unclear, potentially relevant to the age-associated nature of the diseases of interest. Age-associated factors that impact reprogramming efficiency may relate to the epigenetic state of the source somatic cells, the accumulation of genetic mutations in the somatic cells, or alterations in telomere length which have been reported to influence reprogramming efficiency (Wang et al., 2012).

AD: Cell-Type-Specific In Vitro Correlates of Human Brain Pathology

Given the slow progression of all of the neurodegenerative disorders of aging, the notion that these could be modeled in a tissue culture dish has been contentious. But there is accumulating evidence that—despite the typical onset of these diseases late in life—an underlying cellular or molecular pathological process may persist throughout life, particularly in the context of familial inherited disease mutations. This is illustrated in the context of Alzheimer's disease associated with familial mutations in presenilin (PSEN)-1, PSEN-2, or amyloid precursor protein (APP; Israel et al., 2012; Qiang et al., 2011). Mutations in these genes typically cause early-onset forms of Alzheimer's dementia with defects in short-term memory and other realms of cognition, associated pathologically with synaptic and neuronal loss, as well as amyloid plaques and neurofibrillary tangles, in selected brain regions such as the medial temporal lobe. Studies with patient brain tissue and animal models support a role for altered proteolytic processing of APP to the amyloidogenic Aβ42 fragment, relative to the Aβ40 fragment (De Strooper et al., 2012). At a molecular level, PSENs function within the γ-secretase proteolytic complex, and AD-associated familial mutations in PSENs, as well as in APP, modify this intrinsic proteolytic activity so as to increase the relative production of the amyloidogenic Aβ42 form. However, mechanistic questions persist. PSENs are implicated in the processing of several dozen γ-secretase substrates other than APP, and in additional cellular activities such as β-catenin signaling and intracellular endosomal trafficking (De Strooper et al., 2012); the relevance of these functions to human disease remains unclear. Furthermore, it remains unresolved why clinical mutations in PSENs, which are ubiquitously expressed, lead to a selective CNS neuronal degeneration in AD patients. The amyloid hypothesis, that posits a primary role for increased Aβ as necessary and sufficient for AD (Hardy and Selkoe, 2002), remains contentious, in part because therapeutic strategies that specifically target only Aβ have thus far met with limited success in clinic trials.

At least five studies have now pursued hiN or iPSC-based modeling strategies for AD (Israel et al., 2012; Kondo et al., 2013; Qiang et al., 2011; Yagi et al., 2011; Yahata et al., 2011). Directed conversion offers a particularly facile, albeit artificial, approach to pursue cell-type selectivity of a phenotype. Surprisingly, conversion from skin fibroblasts to neurons was found to modify the impact of PSEN mutations on APP processing, in that the relative bias toward production of the pathogenic Aβ42 fragment (relative to Aβ40) appeared magnified in neurons. Why would cellular context impact an intrinsic PSEN γ-secretase activity? Further studies showed that PSEN mutant FAD hiN cultures also displayed altered subcellular localization of APP—toward enlarged endosomal compartments, relative to hiNs from unaffected controls—whereas such redistribution was not apparent in the source skin fibroblasts (Qiang et al., 2011). Relocalization of APP away from the cell surface to endosomes is known to impact APP processing. A similar finding of altered APP localization to endosomes was also described in the context of iPSC-derived neurons from familial AD associated with a duplication of the APP locus (Israel et al., 2012). Of note, the APP cellular relocalization phenotype is not simply a secondary effect of increased Aβ production, as pharmacological blockade of APP processing failed to suppress the modified APP localization. Genetic "rescue" studies, in which wild-type *PSEN1* overexpressed in the PSEN1 mutant hiN cultures suppressed the disease-associated phenotypes, support a direct role for PSEN1 mutation in the phenotype of PSEN1 mutant cells, rather than a spurious effect due to unrelated

Table 3. Human Somatic Cell Reprogramming-Based Neuronal Models of Disease

Disease	Genetic Defect	Drug Testing	Reference
Alzheimer's disease	Duplication of *APP*; Sporadic	no	Israel et al., 2012
	*PSEN1*A246E; *PSEN2*N141I	yes	Yagi et al., 2011
	*APP*E693Δ; *APP*V717L; Sporadic	no	Kondo et al., 2013
Parkinson's disease	α-*synuclein*A53T	no	Soldner et al., 2011
	*LRRK2*G2019S	yes	Cooper et al., 2012; Nguyen et al., 2011; Reinhardt et al., 2013; Sánchez-Danés et al., 2012
	*PINK1*Q456X; V170G	yes	Cooper et al., 2012; Seibler et al., 2011
Spinal muscular atrophy	*SMN* exon7 deletion	yes[a]	Corti et al., 2012; Ebert et al., 2009
Familial amyotrophic lateral sclerosis	*SOD1*L144F	no	Dimos et al., 2008
	*TDP-43*Q343R; M337V; G298S	yes	Egawa et al., 2012
Huntington disease	*Htt 72* CAG repeats	no	An et al., 2012; Zhang et al., 2010
Familial dysautonomia	*IKBKAP* exon20 skipping	yes	Lee and Studer, 2011
Rett syndrome	*MeCP2* mutations	no	Kim et al., 2011d; Marchetto et al., 2010; Xia et al., 2012
Timothy syndrome	*CACNA1C*G406R	yes	Paşca et al., 2011
Gaucher disease	*GCase*N370S/84GG insertion	no	Mazzulli et al., 2011
Spinocerebellar Ataxia Type 2	*ATXN2* (CAG) expansion	no	Xia et al., 2012
Schizophrenia	Sporadic	yes	Brennand et al., 2011

Abbreviations: APP, amyloid precursor protein; PSEN, presenilin; LRRK2, leucine-rich repeat kinase 2; PINK1, PTEN-induced putative kinase 1; SMN, survival of motor neuron protein; SOD1, superoxide dismutase 1; TDP-43, TAR DNA-binding protein-43; Htt, huntingtin; IKBKAP, inhibitor of kappa light polypeptide gene enhancer in B cells, kinase complex-associated protein; MeCP2, methyl CpG binding protein 2; CACNA1C, α-1C subunit of the L-type voltage-gated calcium channel; GCase, glucocerebrosidase; ATXN2, ataxin-2.
[a]Only Ebert et al. performed the drug test.

common variants that may be present in these cultures (Qiang et al., 2011).

These initial studies with human neuron models of familial AD supported the notion that processes other than extracellular Aβ fragment accumulation may play a role in AD pathology. To further address this, Kondo et al. (2013) used human iPSC-derived forebrain cortical neurons that harbor an APP mutation, V717L, also associated with a familial clinical dementia syndrome of the Alzheimer's type, but one that appears to lack the typical amyloid plaques, composed largely of extracellular Aβ42. iPSC-derived neurons from patients with the V717L APP mutation showed reduced extracellular Aβ42 and Aβ40, consistent with the CNS pathology in human patients with this mutation. Interestingly, intracellular accumulation of Aβ forms was increased (Kondo et al., 2013), suggesting an alternative mechanism of pathology. The increase intracellular Aβ was correlated with markers of endoplasmic reticulum (ER) and oxidative stress, as well as apoptosis, in the iPSC-derived neuron cultures carrying the V717L mutation. Docosahexaenoic acid (DHA), a therapeutic candidate for AD, relieved the ER stress responses and suppressed apoptosis in the mutant cells.

An additional pathological finding that typifies AD patient brain is the accumulation of modified, hyperphosphorylated, and aggregated TAU protein, leading to the accumulation of neurofibrillary tangles. APP mutant human iPSC-derived neuron cultures have been reported to harbor increased TAU phosphorylation (Israel et al., 2012), whereas the majority of transgenic rodent models fail to do so, likely reflecting species differences in the *TAU* gene. Interestingly, inhibition of γ-secretase—which is required for Aβ fragment generation—failed to suppress such phospho-TAU pathology in iPSC-derived APP mutant neurons, whereas inhibition of β-secretase function appeared effective (Israel et al., 2012). As inhibition of either secretase complex suppresses Aβ production, this finding further supported the notion that aspects of APP biology other than extracellular Aβ accumulation may play an important role in AD pathology. A central remaining question is whether late-onset nonfamilial AD, which represents the vast majority of cases, is associated with similar cellular and molecular mechanisms. A preliminary analysis of iPSC-derived neuron cultures from two individuals with common nonfamilial AD reported that one of these displayed changes in APP processing akin to those seen in cultures with familial mutations in APP, whereas the second did not show such changes; these data underscore the apparent heterogeneity of common nonfamilial disease and the need for expanded cohorts. Common genetic variants in the human population, such as at the *APOE* and *SORLA/SORL1* loci, significantly impact sporadic AD risk (Bettens et al., 2013), and thus it will be of interest to pursue the impact of such variants in reprogramming-based human cell models of AD. For instance, as SORLA/SORL1 is thought to play a role in the trafficking of APP to and from intracellular endosomal compartments (Rogaeva et al., 2007), it is tempting to consider the functional consequences of human SORLA/SORL1 variants on APP processing in the human reprogramming models.

Table 4. Technologies for the Correction (or Introduction) of Disease-Associated Mutations in Human Cell Models

Genomic Correction Method	Applied Neurological Disorder	Reference
Sequence-specific oligodeoxynucleotides mediated replacement	Spinal muscular atrophy	Corti et al., 2012
Zinc finger nuclease (ZFN)-mediated editing	Parkinson's disease	Reinhardt et al., 2013; Soldner et al., 2011
Transcription activator-like effector nucleases (TALENs) mediated editing	N/A	Hockemeyer et al., 2011
Helper-dependent adenoviral vector (HDAdV)-mediated gene editing	Parkinson's disease	Liu et al., 2012
Homologous arms from a bacterial artificial chromosome (BAC) mediated homologous recombination	Huntington disease	An et al., 2012

PD and the Role of Environmental Stressors

Rodent genetic models of PD have often failed to recapitulate key aspects of human disease pathology, such as the somewhat selective midbrain dopaminergic neuron loss or accumulation of intracellular aggregates of αSynuclein (αSyn) protein (Dawson et al., 2010). This may simply reflect the lengthy of the time course of the human disease, or species-specific aspects. Also, environmental insults such as toxins have been hypothesized to interact with genetic factors in the pathogenesis of PD. Autosomal-dominant mutations in LRRK2, which encodes a large multidomain kinase, represent the most common known familial genetic cause of PD. LRRK2 mutant iPSC-derived neurons from familial PD patients have been associated with increased sensitivity to oxidative stress, such as in the form of 6-hydroxydopamine or 1-methyl-4-phenylpyridinium (MPP^+)—which selectively enter dopaminergic neurons through the dopamine transporter—as well as hydrogen peroxide or rotenone (Cooper et al., 2012; Nguyen et al., 2011; Reinhardt et al., 2013). The increased sensitivity is associated with activation of extracellular signaling-related kinases (ERKs), and inhibition of this pathway ameliorated toxicity (Reinhardt et al., 2013). Similarly, increased sensitivity to oxidative toxins has been reported with iPSC-derived neurons that harbor PD-associated homozygous recessive mutations in PINK1 (Cooper et al., 2012), a mitochondrial kinase, or a familial inherited triplication of the *αSyn* locus (Byers et al., 2011).

The tremendous genetic diversity across the human population does raise the possibility that any given phenotype observed in cultures from a unique individual may not be due to a particular mutation or disease. To link mutations in PD genes to cell phenotypes, an elegant approach is the precise genetic correction of the lesion, as was described for a PD-associated *αSyn* missense mutation using zinc finger nuclease (ZFN) technology (Soldner et al., 2011). There are now several additional effective platforms for genetic correction, summarized in Table 4. Transcription activator-like effector (TALE) nucleases, like ZFNs, allow for the precise correction (or induction) of genomic mutations, so as to enable the subsequent phenotypic analysis of mutant cells alongside isogenic "control" cultures (Ding et al., 2013; Hockemeyer et al., 2011; Soldner et al., 2011). Both technologies introduce DNA nucleases that are fused to DNA-binding protein elements, designed to generate double-stranded DNA breaks at selected genomic sites. These DNA breaks promote homologous recombination with exogenous or endogenous DNA sequences. A limitation with the ZNF and TALE nuclease technologies is that they must be custom-engineered and empirically tested for each desired site in the genome. A more recent approach derived from prokaryotic adaptive immune defenses, termed clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas), enables RNA-guided genomic editing, and is potentially simpler to design (Cong et al., 2013; Mali et al., 2013); CRISPR remains unproven in the context of cell-based disease models. Using yet another approach—helper-dependent adenoviral vector (HDAdV) mediated gene targeting—a recent study "corrected" PD-associated familial mutations in LRRK2 in iPSC cultures, and thereby linked these mutations with alterations in nuclear envelope structure (Liu et al., 2012). It remains to be determined whether such nuclear envelope changes are consistent findings in PD patient-derived cultures.

Studies in human iPSC-derived neuronal models of PD have also sought to reveal mechanistic details about PD etiology, such as mitochondrial alterations (Jiang et al., 2012; Seibler et al., 2011), and how these may lead to the pathological features of the disease. iPSC-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 that is encoded by another familial PD gene (Rakovic et al., 2013). Surprisingly, PARKIN-deficient iPSC-derived neurons from familial PD patients did not appear to show frank mitochondrial defects, suggesting potential redundancy (Jiang et al., 2012). It remains unclear from these studies why dopaminergic neurons are particularly vulnerable to mutations in genes that appear widely expressed, but the iPSC-based models are well-positioned to pursue that issue. One possibility is that dopaminergic neurons are prone to a higher level of intrinsic oxidative stress, which predisposes the cells to damage in the context of PD familial genetic mutations.

In addition to mitochondrial pathology in PD, another prominent feature is the accumulation of αSyn protein, which has been noted to be increased in sporadic disease as well as familial forms. Heterozygous carriers of mutations in β-glucocerebrosidase (GBA), which encodes an essential lysosomal degradation machinery enzyme, are at increased risk of PD, and iPSC derived neurons from such individuals have been reported to display a dramatically increased accumulation of αSyn protein (Mazzulli et al., 2011), as is seen in PD brain pathology. In the homozygous state, GBA mutations are associated with Gaucher's disease, with severe lysosomal dysfunction typically early in life (Mazzulli

et al., 2011). As expected, iPSC derived neurons that harbor triplication of the *αSyn* locus display similarly increased accumulation of αSyn protein (Byers et al., 2011). Regulation of *αSyn* gene expression is species specific, and appears to be modified both in familial and sporadic PD brain (Rhinn et al., 2012); thus in vitro human neuronal models may prove to be particularly useful.

ALS and Nonautonomous Mechanisms of Disease

ALS is characterized by a progressive loss of motor neurons in the spinal cord, leading to difficulty with movement and breathing. Rare familial forms of ALS have been unambiguously associated with mutations in superoxide dismutase-1 (SOD1), transactive response DNA-binding 43 (TDP-43), fused in sarcoma (FUS), C9orf72, and approximately a dozen other genes (Ferraiuolo et al., 2011). A common theme in the context of several of these familial forms—including mutant forms of SOD1, TDP-43, FUS, and C9orf72—is the formation of cytoplasmic aggregates (Ash et al., 2013; Da Cruz and Cleveland, 2011; Mori et al., 2013). Furthermore, TDP-43 aggregates are found in the majority of nonfamilial "sporadic" ALS cases even in the absence of known mutations, supporting the idea that common mechanisms underlie the familial and sporadic forms. Cytoplasmic TDP-43 aggregates are typically seen in neurons and astrocytes along with concurrent "clearing" of the normal nuclear localization of TDP-43, and this has opened the possibility that loss of nuclear TDP-43 function, as well as aggregation, may play a role in pathology.

Model organism studies, from mice to yeast, have brought significant insight into the role of genes such as *TDP-43* in vivo, but questions persist about the specific mechanism of action in the context of human motor neurons. For instance, the relative importance of protein aggregates, nuclear clearing, and the nonautonomous impact of astroglial pathology on motor neuron loss (Da Cruz and Cleveland, 2011) is unclear. Initial analyses of iPSC-derived spinal motor neurons with mutations in TDP-43 have reported evidence of reduced motor neuron survival in vitro, particularly in the context of an oxidative toxin, arsenite, and accumulation of TDP-43 (Bilican et al., 2012; Egawa et al., 2012). A critical point is that these studies did not include validation using a "rescue" approach or cohorts of sufficient size for a statistical analysis, both of which are essential. A cohort of iPSC-derived motor neuron cultures that harbor ALS-associated mutations in SOD1 has been generated (Boulting et al., 2011), and phenotypic analysis with respect to disease-relevant phenotypes will be of interest.

There is broad interest in the role of non-neuronal CNS cell types, such as astrocytes (Lobsiger and Cleveland, 2007), oligodendrocytes (Kang et al., 2013), and microglia (Boillée and Cleveland, 2008), in ALS pathology. This is based in part on pathological examination at autopsy, as well as on elegant rodent studies that have dissected the impact of ALS-associated mutant SOD1, when expressed selectively within different CNS cell populations, on motor neuron loss (Lobsiger and Cleveland, 2007). Human or rodent ESC-derived motor neurons, (Di Giorgio et al., 2008; Di Giorgio et al., 2007; Nagai et al., 2007), as well as human iPSC-derived motor neurons (Serio et al., 2013), have been reported to display reduced survival when co-cultured with murine astrocytes that overexpress mutant SOD1 (as compared to control astrocytes). The nature of the astrocytes -derived factor has been speculated to be a secreted inflammatory mediator (Lobsiger and Cleveland, 2007); an alternative and intriguing concept is that the factor may represent extracellular propagation of the mutant SOD1 protein itself (Pimplikar et al., 2010). A role for astrocytes in ALS pathology has also been considered with respect to TDP-43 mutations (Serio et al., 2013). Taken together, these studies are intriguing but require further validation with additional cultures and using "rescue" approaches. The nonautonomous role of astrocytes and other cell types in CNS neurodegeneration is of interest beyond ALS (Lobsiger and Cleveland, 2007; Polymenidou and Cleveland, 2011), in disorders such as with PD, AD, and frontotemporal dementia (FTD). In vitro coculture approaches offer a reductionist model system to address this mechanism.

Therapeutics: The Endgame

Human reprogramming-based neuronal models offer the potential of "personalized medicine" strategies for adult CNS disorders, wherein neurons from a particular patient would be used to optimize an individualized therapeutic approach. Beyond that, human cells may complement limitations of animal models. A major disappointment over the past decade has been the lack of significant efficacy—in human clinical trials for AD, PD, and ALS—of a host of candidate drugs that had previously appeared potent in animal models. For instance γ-secretase inhibitors, such as semagacestat, are highly effective in transgenic models of AD, but failed in human studies (Karran et al., 2011). This may reflect species differences between mouse and man, or the apparently distinct activity of this compound in the context of high levels of APP substrate, as in transgenic mice. Alternatively, it may be that suppressing APP processing to Aβ may not be sufficient to prevent neurodegeneration in AD, if other defects—such as the alterations in endosomal compartments reported in reprogramming-based cell models (Israel et al., 2012; Qiang et al., 2011)—play a significant role.

Therapeutic studies in reprogramming models have typically used candidate approaches to validate or test selected compounds. In a panel of iPSC-derived dopamine neurons from PD patients with mutations in either LRRK2 or PINK1, the kinase inhibitor GW5074 was reported to protect cultures from the toxicity of valinomycin (a potassium ionophore that induces oxidative stress and thus may mimic environmental stressors in vivo [Cooper et al., 2012]). In AD patient iPSC-derived cortical neurons that harbor duplication of the *APP* locus, β-secretase but not γ-secretase inhibitors were found to suppress an altered TAU phosphorylation phenotype (Israel et al., 2012). A histone acetyltransferase inhibitor, anacardic acid, was reported to be protective in the context of TDP-43 mutant iPSC-derived motor neurons treated with the neurotoxin arsenite (Egawa et al., 2012); anacardic acid was chosen on the basis of its potential to modify gene expression changes observed in the mutant cells. It will be important to further validate these candidates therapeutics in multiple independent cell cultures.

Phenotypic analyses of functional neuronal parameters—such as membrane excitability or synaptic connectivity—have thus far been limited, in the context of reprogramming-based models of

neurodegeneration. Recent studies using iPSC-derived neurons in the context of psychiatric disorders, such as schizophrenia (Brennand et al., 2011) and Timothy syndrome (Paşca et al., 2011; Yazawa and Dolmetsch, 2013), have considered such functional neuronal parameters, and attempted to use these analyses in the pursuit of therapeutics. In iPSC-derived cortical neuron cultures from schizophrenia patients and unaffected controls, synaptic connectivity was evaluated in terms of the *trans*-synaptic spread of a modified, fluorescently tagged rabies virus (Brennand et al., 2011). Such synaptic connectivity appeared reduced in the schizophrenia patient iPSC-derived neurons, relative to iPSC-derived neurons from unaffected individuals. Further studies are needed to determine whether this observation can be generalized to independent patient cohorts with schizophrenia, and with respect to its utility in screening potential drugs (Brennand et al., 2011).

The different reprogramming-based neuronal models discussed above may have unique virtues or limitations in the context of drug screens. iPSC-based models allow for extensive expansion of cells, and thus may be beneficial in a broad high-content screen. A method developed to further facilitate the use of iPSC in high-content drug screens enables the expansion and maintenance of iPSC-derived neural progenitors (Koch et al., 2009; Li et al., 2011; Reinhardt et al., 2013). In contrast to iPSC technology, high-content screening with direct reprogramming-based models requires expansion of the source fibroblast cultures, which is limited by senescence. The use of iNSC technology, as detailed above, may combine the advantages of these two approaches. Finally, reprogramming-derived neurons have been pursued as autologous "replacement" cell therapies, as has been reviewed elsewhere (Nakamura et al., 2012).

Epigenetic Reprogramming and the Etiology of Neurological Disorders: Nature versus Nurture

Reprogramming technologies, such as iPSC or iN generation, theoretically "erase" the existing epigenetic state of a cell and establish an alternative state. Such epigenetic states are determined in part by direct modifications of genomic DNA, including methylation or hydroxymethylation, as well as by binding of chromatin factors such as histones that modify the accessibility of genomic DNA (Tomazou and Meissner, 2010). Yet other regulators, that include both protein and non-coding RNA factors, serve to refine the epigenetic state of individual genetic loci. Additionally, the three-dimensional structure of chromatin, determined by yet poorly defined nuclear elements, may broadly impact the epigenetic program.

In the context of patient-derived cultures, historical events of potential relevance to disease—such as aging or toxin exposure—may theoretically underlie a persistent change in epigenetic state, and this may in turn impact cellular phenotypes. The cell-type-specific epigenetic state of a starting cell—in contrast to genetic factors—is predicted to be "erased" in the context of somatic cell reprogramming. Thus, epigenetic reprogramming models, such as patient iPSC-derived neurons, may not display a given disease phenotype, if it is epigenetic in origin. Conversely, a disease-associated phenotype that is apparent in reprogramming-derived cell models is predicted to be genetic in origin. A caveat is that reprogramming has often appeared incomplete: "epigenetic memory" persists in iPSC-derived cultures as to their cells of origin (Kim et al., 2010, 2011c) as well as with directed reprogramming (Khachatryan et al., 2011).

Going forward, it will be of high interest to directly assess epigenetic changes associated with disease states in reprogrammed neuron models. In some contexts, "incomplete reprogramming—which retains significant epigenetic memory—may be desirable. More speculatively, directed reprogramming to neurons may present an advantage over iPSC reprogramming followed by differentiation; single step reprogramming to neurons is perhaps more likely to retain epigenetic memory of prior events, leading to disease-related cellular phenotypes. However, epigenetic memory in skin cells may not be relevant to CNS disorders.

Conclusion

In summary, the application of reprogramming technologies toward the generation of accurate and simple human cell models of adult neurological disorders is a promising approach. It is perhaps unexpected that diseases of aging such as familial Alzheimer's disease would be recapitulated to some extent "in a dish." This reflects an emerging theme, in which underlying molecular and cellular culprits to these diseases of aging may often be present throughout life, whereas unknown "second hits" ultimately lead to the full expression of disease. Cell models may allow for the identification of extrinsic factors that promote the expression of disease phenotypes, as well as ones that suppress such expression.

Reprogramming-based cell models afford a valuable potential approach to the investigation of adult neurological disorders. Although this review focuses on AD, PD, and ALS, many other neurological disorders—such as FTD (Almeida et al., 2012) or susceptibility to herpes simplex virus-I encephalopathy (Lafaille et al., 2012)—are amenable to these approaches. A particularly exciting direction is the application of this technology to the study of non-familial disease, and risk-associated variants. The advent of affordable whole-genome sequencing, as well as large scale genome-wide association studies, are particularly timely in this regard. A major hurdle to the interpretation of human reprogramming-based disease models is the inherent variation among samples, due both to genetic diversity as well as the distinct personal histories that may lead to epigenetic diversity. It will be essential to use patient and control cohorts (of independent cultures) that are sufficiently large to enable statistically meaningful analyses, which has often not been the case in "first-generation" models. Furthermore, going forward, studies that lack a genetic or biochemical complementation approach to directly link a given genetic variant (or mutation) a phenotype must be treated with some skepticism.

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