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Impact of induced pluripotent stem cells on the study of central nervous system disease

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Abstract

The derivation of pluripotent stem cells from somatic tissues has provided researchers with a source of patient-specific stem cells. The potential applications of this technology are truly momentous, and include cellular modeling of disease processes, drug discovery, and cell-based therapy. Here, we review the use of induced pluripotent stem cells (iPSCs) to study CNS disease. Since the iPSC field is still in its infancy, we also discuss some of the challenges that will need to be overcome before the potential of this technology to study and to treat neurological and psychiatric disorders can be fully harnessed.

Introduction

Advances on the molecular underpinnings of brain disease have evolved slowly relative to other organs. The reasons for this lag are many, such as the intrinsic complexity and the highly extended time course of brain development that, for critical events such as synaptic pruning, neuronal maturation, and axon myelination, extends into the second and third decades of life. An additional challenge to brain disease research is the simple fact that, for most purposes, biopsy of diseased tissue is not an option.

While imaging and electrophysiological techniques have made amazing progress in understanding systems-levels aspects of human brain development and function, discovering the molecular causes of brain disease requires methods that can evaluate the functions and interactions of specific molecules within distinctly identifiable types of cells. Of course, animal models continue to provide a wealth of information critical to understanding brain disease, but these have inherent limitations. Recently, the ability to generate conceivably any cell type from human embryonic stem cells has opened up a new era in the study, and perhaps even the treatment, of brain disease [1]. The advent of technology for the derivation of induced pluripotent stem cell (iPSC) lines, whereby self-renewing, pluripotent stem cells are generated from somatic cells, expands this era into the realm of patient-derived stem cells [2,3]. By directing iPSCs into disease-relevant cell types, there is finally an approach for conducting biopsy-like experiments on living tissue from diseased individuals, with the added capacity to study the initial development and progression of pathology (Figure 1). Here, we briefly review progress in the modeling of neurological disease through the use of iPSCs. Opportunities and challenges associated with extending this progress to the realm of

neuropsychiatric disorders are then presented. For comprehensive reviews related to establishing pluripotency and generating tissues, the authors recommend [4,5].

The dawn of a new era: Induced Pluripotent Stem cells (iPSCs)

In 2007, Yamanaka and colleagues successfully derived pluripotent stem cells from human fibroblasts, marking the first time somatic conversion to embryonic-stem cell state had been achieved [2,3]. These human `induced' pluripotent stem cells are akin to human embryonic stem cells (hESCs), possessing to ability to maintain pluripotency and to generate all three germ layer lineages. In the Yamanaka *et al*. study, they essentially re-set the fibroblasts to a pseudo-embryonic stem cell state by transducing them with four transcription factors, Oct4, Sox2, Klf4, and c-Myc using a retroviral approach. Since this pioneering study, the iPSC field has grown rapidly as researchers refine the process of directed reprogramming. Due to concerns associated with viral integration, including the possibility of oncogene reactivation and the generation of insertional mutations, the field has focused recently on developing integration-free methods. Some of these include transfection of episomal plasmids, non-integrating viral vectors including the sendai virus, small molecule inhibitors, and synthetic RNAs (Figure 1) [3,6–15]. While the non-integrating approaches hold certain advantages, the reprogramming approach one chooses will ultimately depend on the experimental goal and available resources. For example, due to their greater efficiency of reprogramming (an attribute that strongly affects costs), and to their ease of including the expression of marker protein for identifying iPSC-derived cells in transplant experiments, lentiviral vectors that contain the reprogramming factors in a single construct are likely to be a mainstay of preclinical iPSC studies for at least the near-term future.

Disease-specific lines: challenges and potential

The application of iPSC technology to the study brain disorders requires three fundamental steps. First, the elaboration of goals or hypotheses; is the intent to derive relevant tissues from individuals with a given disorder, then screen for alterations of signaling systems that may point to causative underpinnings and/or to foci for treatment? Is the goal to test specific hypotheses on the nature of the disorder? Such considerations can impact key decisions on subjects and controls to be studied, source cell type to be harvested, and reprogramming method to be applied. Second, the derivation of iPSC lines from individuals who suffer from a disorder. Third, the directed differentiation of these lines to neural cell types that may be relevant to the disorder. The likelihood of accomplishing the second two steps in a manner that is meaningful to the first one revolve around several key issues, including the genetics and penetrance of the disorder and the availability of protocols to derive the relevant cell types (Table 1). Hence, the disease or disorders currently most amenable to study by iPSC technology are those that involve single gene (or single chromosomal) abnormalities that result in highly penetrant phenotypes. In addition, since the study of a neural phenotype will require either a cell culture or xenograft transplantation approach, disorders most amenable to study will be those for which a phenotype is present relatively early in development.

Monogenic, early-onset neurological disorders

Recently, several groups have generated disease-specific lines form patients with neurodevelopmental/neurogenetic diseases including Rett Syndrome [16,17], Fragile X syndrome [18], Prader-Wiilli/Angelman syndrome [19], Spinal Muscular Dystrophy (SMA) [20,21], Familial Dysautonomia (FD) [22], and Downs Syndrome [23] (Table 2).

Rett Syndrome

Rett syndrome is a X-linked autism-spectrum neurodevelopmental disorder caused by mutation(s) in the methyl CpG-binding protein (MECP2) [24–27]. In 2009, Hotta *et al*. derived an iPSC line from an 8-year old Rett patient possessing the heterozygous R306C missense mutation in MECP2, which disrupts with normal neuronal maturation. MECP2 binds to methylated DNA, thus its function is directly related to epigenetic status. During reprogramming there is large scale, (but probably incomplete [28]) erasure of epigenetic marks [29,30]. Whether and how this might interfere with studying a gene that modulates the chromatin structure is not clear. However, evidence that selective loss of MECP2 in forebrain GABAergic neurons can phenocopy aspects of autism and Rett's disorder, [31] enhances the likelihood that an iPSC-mediated approach will shed useful light on this disorder.

Prader-Willi and Angelman Syndromes

Chamberlain *et al*. [19] recently derived iPSCs from Angelman (AS) and Prader-Willi (PWS) Syndrome patients, both of which are neurodevelopmental disorders of genomic imprinting. Importantly, in this study the authors describe an *in vitro* system for AS, measuring AMPA-receptor-mediated spontaneous activity, providing a platform to study disease mechanism of AS in future studies using AS-iPSCs. Additionally, the PW-iPSC lines show no disrupted methylamine patterns in the `Prader-Willi imprinting center (PWS-IC) reprogrammed cells compared to the source fibroblast lines. This study indicated that genomic imprinting can be refractory to the epigenetic erasure produced during reprogramming.

Fragile X

Further evidence that the iPSC reprogramming process doesn't necessarily impinge on the epigenomic status contributing to a diseased state comes from a set of iPSC lines derived from patients with Fragile X syndrome. Fragile X is a common form of mental retardation characterized by a lack of expression of the *FMR1*, a gene that is normally expressed during the ESC state with silencing during ESC differentiation. Urbach *et al*. [18] recently derived three iPSC lines from males affected with Fragile X (FX-iPSCs). This study reports that the mutant FMR locus in FX-iPSCs is not `re-set' during the reprogramming process. However, a key finding within this study showed that in general, there are significant epigenetic differences between hESCs and hiPSCS, which may or may not prove to be a limitation of using patient-specific iPSC lines and hESCs in comparative studies.

Familial Dysautonomia (FD)

Currently, the most promising use of iPSC technology to study disease pathogenesis *in vitro* is with the iPSC lines derived from FD patients. FD is an inherited, autosomal recessive disorder characterized by progressive deterioration of the autonomic and sensory neurons. In the case of FD, the observed phenotype has been attributed to the predominant mutation associated with FD, a reduction in splicing efficiency of IKBKAP [32]. In 2009, Lee *et al*. derived iPSC lines from FD patients and demonstrated that FD-iPSC derived neural crest precursors exhibited reduced splicing efficiency of IKBKAP, a decrease in neurogenesis, and reduced neuronal migration. This study is a rare case, but probably the first of many, in which the iPSC lines were used to test a potential treatment, as addition of splicing inhibitors could partially normalize the disease phenotype.

Spinal Muscular Atrophy (SMA)

Similar to FD, SMA exhibits an *in vitro* phenotype, notably a decrease in motor neuron survival. SMA is an autosomal recessive neurogenetic disorder caused by mutations in the

SMN1 gene resulting in marked reduction in SMN1 protein expression and progressive loss of motor neurons. Ebert *et al*. [20] generated iPSCs from patients with Type I SMA containing partial deletions of the *SMN1* gene. *In vitro* survival studies demonstrate that there is a progressive loss of the motor neurons in culture.

It is important to point out that one of the potential uses of iPSCs is to use these cells to identify new drug targets and screen for off-target toxicities of lead compounds. In the case of drug screening and presumably, setting up high throughput drug screens, it is necessary to have a robust *in vitro* phenotype to assay, which in the case of some of the neurodevelopmental disorders described, appears to be a realistic, achievable goal.

Polygenic, late-onset Disorders

Neurodegenerative Disorders

Disease-relevant cell types and disease-specific iPSCs have been generated from patients with many of the neurodegenerative diseases including Alzheimer's, Parkinson's disease [33] [23], Amytrophic Lateral Sclerosis (ALS) [34], and Huntington's Disease [23] (Table 2). Although there has been a large focus on generating many lines, however, the overriding issue in all of these disorders is the lack of a defined *in vitro* phenotype with each disease-relevant sub-type generated. Despite this progress, it is seemingly useless to model disease of these complex disorders with a lack of defined phenotype in a dish. In order for iPSCs cells to prove useful for human disease modeling, *in vitro* cell conditions must more accurately recapitulate the environments contributing to the sporadic forms of the diseases. For example, oxidative stress may play an important role in the etiology of sporadic PD [35,36], suggesting that patient-derived midbrain dopaminergic cells may show increased vulnerability to oxidative stress.

Alzheimer's Disease (AD)

Although no reports have been published detailing derivation of patient-specific iPSC associated with Alzheimer's, a in-depth article on the Alzheimer's Research forum website http://www.alzforum.org/, reports that efforts are currently underway to generate various lines from patients with various sporadic and familial dementias, including AD and front temporal dementia (Part 1, http://www.alzforum.org/new/detail.asp?id=2558). Collectively, researchers in the field have created lines from patients harboring amyloid precursor protein duplications, preselenin-1, and tau mutations. These efforts are part of larger consortia aimed building a compendium of iPSC lines form both sporadic and familial forms of AD. Due to the protracted delay to onset of AD pathology, and the lack of a specific cell type involved (i.e. a particular neuron class within a particular brain structure, although cholinergic cells of *N. Basalis* are a logical initial target), application of iPSC technology to AD may be particularly difficult.

Parkinson's Disease (PD)

Perhaps the greatest effort in creating iPSC lines has been within the field of PD. The list of patient-specific lines created is rapidly expanding with groups deriving lines from patients with alpha-synuclein triplications, mutations in LRRK2, and mutations in SCNA, as well as from patients with sporadic forms of PD. Isacson *et al*. [37] have successfully derived iPSCs from patients with idiopathic PD, differentiated the cells into functional dopaminergic (DA) neurons, and performed xenografts into adult rodent striatum. However, this monumental study is the first account of using human iPSCs for xenograft transplantation studies in an animal model of PD. As promising as this study is, no phenotype was evident in the PD-derived cells, thus highlighting the challenge of studying late-onset human disease in this manner.

Amyotrophic Lateral Sclerosis (ALS)

Similar to PD, a large-scale consortium headed by both groups at the Harvard Stem Cell Institute (HSCI) and Johns Hopkins University is focused on creating and cataloging iPSC lines from both patients with both sporadic and familial ALS, a neurodegenerative disorder affecting motor neurons. However, like PD and AD, a disease-related phenotype such as neuronal inclusions or increased apoptosis has yet to be detected.

iPSC Consortia

A useful resource for researchers studying neurodegenerative disorders interested in patient-specific fibroblast or iPSC lines is the NINDS/Coriell Cell bank repository which receives, stores, and standardizes the collection of iPSC lines related to ALS, PD, and HD (http://ccr.coriell.org/sections/collections/NINDS/?SsId=10). The objective of this consortium is to generate iPSC lines from all familial forms of the 3 major neurodegenerative disorders with inclusion of cell-specific reporters associated with each disorders. The Harvard Stem Cell Institute (HSCI) iPSC core is also cataloging lines produced by HSCI scientists and provides a service to produce disease-specific lines. (http://www.hsci.harvard.edu/node/1005).

Neuropsychiatric disorders

Neuropsychiatric disorders such as schizophrenia and autism appear to largely result from the combinatorial effects of polygenic risk factors with a significant component of environmental influence (probably mainly *in utero*), techniques for addressing this complicated intermix of effects would be tremendously useful. Although at the time of writing this review we are aware of no published reports of iPSC lines derived from patients affected with schizophrenia or autism, given the number of groups that have obtained funding for making such lines publications are likely to be forthcoming. Particular challenges to using iPSCs to study complex disorders, those in which gene-gene and gene-environmental interactions result in considerable variability in the presentation of symptoms, lies in the variability that may be inherent to the reprogramming process. For example, a recent report found that comparison of iPSC lines from the same source material, directed by the same protocol, could show wide variation in the expression of Pax6, an early marker of neuropithelium [38]. It will thus be critical that whatever measure is being used to compare neural cells derived from patient and controls, the variability of that measure within multiple lines derived from the same source must be known.

Summary: Impact of iPSC technology on the study of neurological and psychiatric disorders

At the moment, the iPSC approach to studying brain disease remains one, overall, of tremendous promise. However, these are clearly “early days”, and many challenges will need to be overcome before mechanistic insights for major brain diseases, such as Alzheimer's disease, schizophrenia, or autism, are generated via iPSC approaches. Perhaps the most critical challenge lies not within the derivation from a given patient and control group of reasonably uniform lines of pluripotent cells in a cost-effected manner, but developing the cell culture and xenograft approaches that will be required to use these lines for the study of brain disease.

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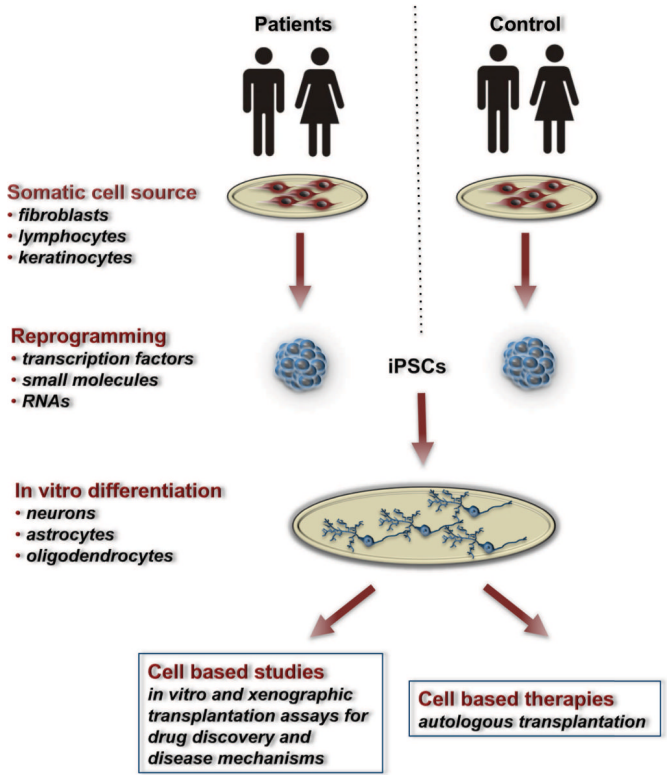

Figure 1. Using human iPSCs to study and treat neurological and psychiatric disorders

Somatic cells from patients or controls can be harvested from several tissue types (i.e. fibroblasts, lymphocytes, keratinocytes) and reprogrammed into iPSCs using ectopic expression of transcription factors, small molecule inhibitors, or synthetic modified-RNAs. Patient-specific pluripotent stem cells can then be directed to disease-relevant cell types for use in mechanistic studies and cell-based therapies. Examples of these could be myelinating oligodendrocytes for multiple sclerosis or ALS, or GABAergic interneurons for seizure disorders [73] or Parkinson's disease [74].

Table 1

Derivation of CNS cell types from Pluripotent Stem Cells (mouse* and human**)

CNS Cell Type	References
Dorsal forebrain precursors/neurons	[39–44]* [45]**
Ventral forebrain precursors/neurons	[46–48]*
Rostral-hypothalamic neurons	[49]*
Midbrain dopaminergic neurons	[50]* [51–55]**
Cerebellar precursors/neurons	[56,57]* [58]**
Spinal motor neurons	[59–62]**
Oligodendrocytes	[63–65]* [66]**

Table 2

Summary of iPSC lines

Neurological Disease	Neuronal Population(s) Primarily Affected	Genetics of Inheritance	Mutated gene/protein associated with familial forms	iPSC line comments
Rett Syndrome	Differentiated neurons [67,68]	X-linked dominant De novo: ~95% Germline-Xq28	*MeCP2:* several mutations	• Derived and differentiated into neurons [16] • Observed *in vitro* phenotype-reduced spine density, altered calcium signaling [17]
Prader-Willi Syndrome (PWS)	Hypothalamic neurons [69]	X-linked imprinting	15q11-q13 paternal deletion	• PWS-iPSCs: No disruption of imprinting center [19]
Angelman Syndrome (AS)	Global: Regulation of spine development and synaptic plasticity [70] Hippocampal and cerebellar neurons [71]	Maternal imprinting	15q11-q13 maternal deletion *UBE3A*	• Potential *in vitro* system: measuring AMPA-receptor mediated activity [19]
Fragile X	Global	X-linked dominant	*FMR1* CGG repeat truncation	• [18]
Familial Dysautonomia (FD)	Sensory and autonomic neurons	Autosomal recessive	*lKβK4P* q19 T-C intron 20 most common	• FD-iPSCs: • Reduced IK(3KAP splicing efficency [22]
Spinal Muscular Atrophy (SMA)	Motor neurons	Autosomal recessive	*SMN2*	• SMA-iPSCS: • Progressive loss of motor neuron survival [20]
Downs' syndrome	Global		Trisomy 21	• No *in vitro* phenotype indentified
Alzheimer's Disease (AD)	Cortical neurons Hippocampal neurons	Dominant	Aβ precursor protein Apolipoprotein E Preselenin 1,2	• No *in vitro* phenotype identified
Parkinson's Disease (PD)	DA neurons	Autosomal dominant/recessive	*LRRK2* *PARK 1 – 11*	• PD-iPSCs xenografted into animal model of PD; • No *in vitro* pheotype identified

Neurological Disease	Neuronal Population(s) Primarily Affected	Genetics of Inheritance	Mutated gene/protein associated with familial forms	iPSC line comments
				• Several Lines generated • [23,33,37]
Amyotrophic Lateral Sclerosis (ALS)	Motor neurons	Autosomal dominant	*SOD1*	• No *in vitro* phenotype identified [72]
Huntington's Disease (HD)	Cortical-striatal neurons	Autosomal dominant	*HTT*	• No *in vitro* phenotype identified • Several lines in queue (Coriell)