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Current Opinion in Neurobiology

Modeling neurodevelopmental disorders using human neurons

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The cellular and molecular mechanisms of neurodevelopmental conditions such as autism spectrum disorders have been studied intensively for decades. The unavailability of live patient neurons for research, however, has represented a major obstacle in the elucidation of the disease etiologies. Recently, the development of induced pluripotent stem cell (iPSC) technology allows for the generation of human neurons from somatic cells of patients. We review ongoing studies using iPSCs as an approach to model neurodevelopmental disorders, the promise and caveats of this technique and its potential for drug screening. The reproducible findings of relevant phenotypes in Rett syndrome iPSC-derived neurons suggest that iPSC technology offers a novel and unique opportunity for the understanding of and the development of therapeutics for other autism spectrum disorders.

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Current Opinion in Neurobiology 2012, **22**:785–790

This review comes from a themed issue on **Neurodevelopment and disease**

Edited by **Joseph Gleeson** and **Franck Polleux**

For a complete overview see the Issue and the Editorial

Available online 19th June 2012

0959-4388/$ – see front matter, Published by Elsevier Ltd.

http://dx.doi.org/10.1016/j.conb.2012.04.004

Introduction

The limited potential of neuronal samples from postmortem brains and the inability to isolate populations of neurons from living subjects has blocked progress toward understanding the cellular and molecular mechanisms behind several neurodevelopmental disorders. Studies of postmortem tissue are problematic in developmental disorders as disease onset usually precedes death by decades. Moreover, frozen tissue sections are of limited use for studying cellular physiology and neural networks. Peripheral tissues, such as blood, are not suitable for relevant biological experiments since they are not the target tissue. Mathematical or computational models are also restricted by nature. Brain imaging allows you to study circuitries at a low magnification and does not reveal details of short circuitries in the brain. Finally, animal models often do not recapitulate complex human diseases, and have been particularly problematic in the case of human neurodevelopmental disease such as autism. Thus, the field lacks a human model that could provide unlimited supplies of neurons so experiments can be performed in controlled situations.

Genetic reprogramming provides a complementary model as it allows the genomes of human individuals afflicted with neurodevelopmental diseases to be captured in a pluripotent stem cell line. Reprogramming of somatic cells to a pluripotent state by overexpression of specific genes has been accomplished using mouse and human cells [1,2••]. These reprogrammed cell types, named induced pluripotent stem cells (iPSCs) can be derived from cells isolated from peripheral tissues of normal individuals or people affected from several conditions [3]. Isogenic pluripotent cells are attractive not only for their potential therapeutic use with lower risk of immune rejection, but also for their prospects to further understanding of complex diseases with heritable and sporadic conditions [4,5]. iPSCs can then be differentiated to human neurons to evaluate whether the captured genome alters cellular phenotypes in a similar manner as predicted by the clinical data or other mechanistic models. Although iPSCs have been generated for several neurological diseases the demonstration of disease-specific pathogenesis and phenotypic rescue in relevant cell types is a current challenge in the field, with only a handful proof-of-principle examples to date [6]. Nonetheless, the examples reflect the potential that this new model brings to disease modeling.

Considerations about iPSCs as a model for neurodevelopmental diseases

As with other models, the iPSC system also has important limitations. Cells in culture represent a research artifact. Thus, it is possible that important signaling information is missing or overstimulated in the system, masking potential cellular phenotypes or creating artificial ones. The discrimination between what is real and truly important *in vivo* will probably depend on validations coming from other models. Another challenge is the derivation of relevant neuronal subtypes. Specific protocols for subtypes of neurons are currently being developed and need further optimization. Alternatively, the relevant neuronal subtype needs to be sorted out or visualized using specific reporter genes. Unfortunately, the characterization of human neuronal subtypes is modest owing to the lack of knowledge on the temporal expression of specific genes and respective promoter activation. Recent efforts on human brain expression maps will certainly help [7,8].

Another important consideration is the use of appropriate controls. Intuitively, the ideal controls are the ones that differ from the patient by only the specific genetic defect. The targeted manipulation of the iPSCs to introduce genetic mutations in control cell lines or to restore the mutation from a patient cell line is a promising tool [9,10]. Another strategy to generate 'isogenic' cell lines is to take advantage of X-inactivation in iPSC clones coming from female cell lines. Owing to the fast X inactivation process during reprogramming, it is possible to generate iPSC cell clones carrying the mutant or the wild-type version of a X-linked affected gene. That strategy was used to model Rett syndrome, using female patients with mutations in the X-linked MeCP2 gene [11••,12]. For non-monogenetic diseases, or when the mutations are not known, such as sporadic autism, the challenge is greater. Behavioral variation between cell lines and iPSC clones from the same individual can influence phenotypic readouts. Unfortunately, the generation of individual iPSC clones is also expensive and time-consuming, restricting the number of cell lines that an individual laboratory can handle. A possible useful strategy for these types of disease is the coordination of consortium initiatives, where multiple sites would contribute to the pool of different cell types and phenotypic assays. Nonetheless, methods for generating neurons at a large scale and automated phenotypic analyses will become essential to realize such an endeavor.

Modeling neurodevelopmental diseases in a dish

A few years after the success of somatic cell reprogramming was reported in 2006, iPSC technology has been extensively used to model several neurodevelopmental diseases including a monogenic form of autism spectrum disorders (ASDs). Rett syndrome (RTT) [11••,12,13,15], sporadic form of Schizophrenia (SCZD) [16•], fragile X syndrome (FXS) [17], and Timothy syndrome (TS) [18••]. These studies were able to demonstrate that such disease-specific iPSC-derived neurons elegantly recapitulated relevant cellular and/or molecular phenotypes previously reported using different approaches, for example, postmortem human brain, and mouse model. In addition to those disorders, somatic cell reprogramming has recently been conducted for modeling another early onset neurological diseases including CDKL5-related disorder (a RTT variant) [19], and genomic imprinting disorders such as Angelman syndrome (AS) [20] and Prader-Willi syndrome (PWS) [20,21]. Successful neuronal differentiation was shown in these studies but the phenotypes of such disease-specific iPSC-derived neurons have not yet been examined. The neurodevelopmental diseases that have been modeling using human iPSC thus far and their significant findings are summarized in Table 1.

Modeling Rett syndrome with iPSCs

We recently demonstrated the utility of induced pluripotent stem cells to investigate the functional consequences of mutations in the gene encoding the Methyl CpG binding protein-2 (MeCP2) on neurons from RTT patients, a syndromic form of ASD [11]. RTT patients appear to develop normally for up to 6–18 months, after which they enter a period of regression characterized by deceleration of head growth and loss of acquired motor and language skills. Patients often develop autistic behaviors, stereotypic hand wringing, abnormal breathing and seizures [22]. Postmortem analyses of affected individuals have revealed phenotypes at the cellular level, including decreased soma size, reduced dendritic branching, and altered dendritic spine density and morphology [23,24].

MeCP2 is an abundant nuclear protein implicated in a number of molecular functions, but was first identified as an epigenetic regulator of target genes by binding to methylated CpG dinucleotides [25]. Long established as a repressor of transcription, MeCP2 has recently been shown to bind active genes as well as to influence RNA splicing [22,26]. MeCP2 is found in a wide variety of tissues but appears to be most abundant in the brain [27]. Accordingly, MeCP2 appears to be critical for normal CNS development and function, and its dysfunction results in abnormal neurological phenotypes (Figure 1).

Neurons derived from RTT-iPSCs carrying four different MeCP2 mutations showed several alterations compared to five healthy non-affected individuals, such as decreased soma size, altered dendritic spine density and reduced excitatory synapses. These phenotypes were validated using wild-type MeCP2 cDNA and specific shRNAs against MeCP2 in gain-and-loss of function experiments to demonstrate causality. Importantly, some of these cellular defects were immediately validated by independent groups, revealing the robustness and reproducibility of the system [12,13,15]. We were able to rescue the defects in the number of glutamatergic synapses using two candidate drugs, insulin-like growth factor 1 (IGF1) and gentamicin. IGF1 is considered to be a candidate for pharmacological treatment of RTT and potentially other CNS disorders in ongoing clinical trials [28]. Gentamicin, a read-through drug, was also used to rescue neurons carrying a nonsense MeCP2 mutation, by elevating the amount of MeCP2 protein.

Moreover, we took advantage of the RTT-iPSCs to demonstrate that neural progenitor cells carrying MeCP2 mutations have increased susceptibility for L1 retrotransposition. Long interspersed nuclear elements-1 (LINE-1 or L1s) are abundant retrotransposons that comprise approximately 20% of mammalian genomes [29–31] and are highly active in the nervous system [32•,33]. Our data demonstrate that L1 retrotransposition can be controlled in a tissue-specific manner and that disease-related genetic mutations can influence the frequency of neuronal L1 retrotransposition [34]. The work revealed an unexpected and novel phenomenon, adding a new

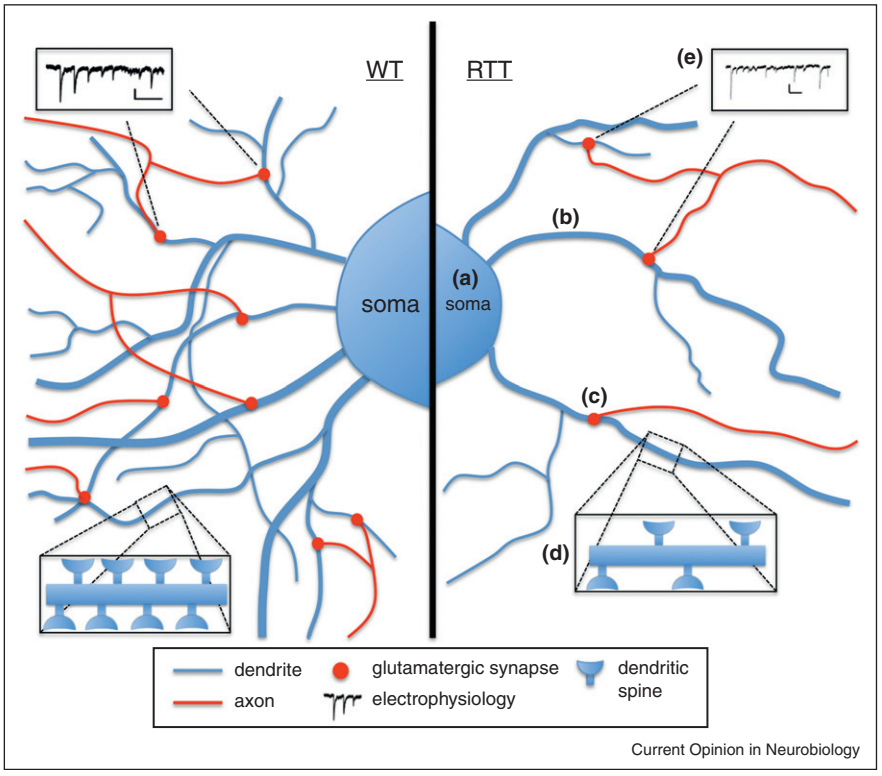
Table 1

Examples of neurodevelopmental diseases that have been modeling using human iPSC

Disease	Incidence	Age of neuropathological onset	Key gene (chromosome)	Genetic mutation in fibroblasts used for reprogramming	Reprogramming method	Neuronal differentiation and validation	Relevant neuronal phenotype	Candidate drug	Reference
RTT	1:10,000 (female) [22]	½–1½ years old [22]	MeCP2 (X)	Nonsense (Q244X) Missense (T158M, R306C)	Retrovirus (4 factors)	Yes; TuJ1+, MAP2+, GABA+, Synapsin+, VGLUT1+ Electrophysiologically active	Reduced soma size, dendritic spine density and synapses Altered Ca^{2+} signaling electrophysiological defect	IGF1	Marchetto [11••]
				Null (Δexon3-4) Missense (T158M, R306C)	Retrovirus (4 factors)	Yes; MAP2+	Reduced soma size	No	Cheung [12]
				Nonsense (Q244X) Missense (T158M, R306C)	Retrovirus (4 factors)	Yes; TuJ1+, SCN1A/B+	Lower expression of mature neuron markers	No	Kim [15]
				Nonsense (V247X, R294X) Missense (T158M, R306C)	Retrovirus and Lentivirus (4 factors)	Yes; TuJ1+	Reduced nuclear size	No	Ananiev [13]
FXS	1:4000–1:6000 [36]	<3 years old [36]	FMR1 (X)	>200 CGG repeats in 5′UTR	Retrovirus (4 factors)	No	No	No	Urbach [37••]
				>700 CGG repeats in 5′UTR	Retrovirus (4 factors)	Yes; TuJ1+	Fewer and shorter neurites	No	Sheridan [17]
SCZD	1:100 [38]	Typically 15–25 years old In rare case, <10 years old [39]	DISC1 (1)	Δexon-intron12 region (4bp)	Integration-free episomes (4 factors)	No	No	No	Chiang [40]
			Sporadic	Unknown	Tetracyclin-inducible lentivirus (5 factors)	Yes; TuJ1+, MAP2+, PSD95+, VGLUT1+, GAD65/67+ Electrophysiologically active	Reduced neuronal connectivity and neurite number, decreased PSD-95 expression	Loxapine	Brennand [16•]
TS	Unknown (20 cases reported worldwide [41])	>2.5 years old [41,42]	CACNA1C (12)	Missense (G406R)	Retrovirus (4 factors)	Yes; MAP2+, VGLUT1/2+, TH+, GAD65/67+, CTIP2+, FOXP1+, SATB2+, Electrophysiologically active	Defect in Ca^{2+} signaling and electrophysiology, decreased SATB2 expression [§]Higher expression of TH and catecholamines	Roscovitine	Pasca [18••]
CDKL5-related disorder	Unknown (80 cases reported worldwide [43])	2–3 months old [44]	CDKL5 (X)	Nonsense (Q347X) Missense (T288I)	Retrovirus (4 factors)	Yes; TuJ1+, MAP2+, VGLUT1/2+, GAD65/67+	No	No	Amenduni [19]
AS	1:12,000 [45]	2–3 years old [46]	Maternal UBE3A (15)	Maternal Δ15q11-q13 (including UBE3A)	Retrovirus (5 factors)	Yes; TuJ1+, MAP2+, SynapsinI+, PanNav+ Electrophysiologically active	No	No	Chamberlain [20]
PWS	1:15,000 [47]	2–6 years old [48]	Unknown in paternal 15q11-q13 (15)	Paternal Δ15q11-q13	Retrovirus (5 factors)	No	No	No	Chamberlain [20]
				t(15;4)(q11.2;q27)	Retrovirus (4 factors)	Yes; TuJ1+, MAP2+	No	No	Yang [21]

4 factors, OCT4, SOX2, KLF4 and c-MYC; 5 factors, OCT4, SOX2, KLF4, c-MYC and LIN28; [§], not observed in animal.

Figure 1



Phenotypes demonstrated by human iPSC-derived RTT neurons. We generated a human cellular model for RTT by reprogramming the fibroblasts of human patients to iPSCs. Neurons differentiated from these iPSCs exhibited cellular phenotypes such as **(a)** smaller soma size, **(b)** reduced dendritic branching, and **(c)** fewer glutamatergic synapses and **(d)** dendritic spines. These morphological alterations contributed to the functional phenotype of **(e)** altered electrophysiology, as RTT-neurons demonstrated a decreased frequency of spontaneous postsynaptic currents.

layer of complexity to the understanding of genomic plasticity. These observations bring valued information for RTT and, potentially, other ASD patients, since they suggest that pre-symptomatic defects may represent novel biomarkers to be exploited as diagnostic tools. The data also suggest that early intervention may be beneficial.

Using human neurons as a drug-screening platform

Our studies performed in RTT highlighted the potential of iPSC models in toxicology and drug screening. Even better, the IGF1 overcorrection observed in some RTT neurons [11••] indicate that the iPSC technology not only can recapitulate some aspects of a genetic disease but also can be used to better design and anticipate results from translational medicine. This cellular model has the potential to lead to the discovery of new compounds to treat different neurodevelopmental diseases.

Drug-screening platforms require 'screenable' robust phenotypes in target cell types, such as iPSC-derived neurons. The neuronal differentiation strategies reported to date are not current capable of providing vast numbers of homogenous subtypes of neurons in a reliable, reproducible, and cost-effective fashion. However, with better markers and gene reporters, it will be possible to isolate pure populations of desired cell types in a large scale. Cellular morphology, such as soma size or dendritic spine density, can be captured using high-content imaging software. Early biochemical and gene expression read outs can be useful alternatives. However, late read outs, such as electrophysiological records, may not be ideal owing to the time in culture necessary to reach neuronal maturation. It is certainly possible to use stressors or other environmental agents to enhance the differences between control and patient groups. An alternative solution may emerge from the direct conversion of neurons from peripheral cells, skipping the pluripotent state [35]. This technology is currently inefficient in humans, difficult to scale up and has the disadvantage to skip neuronal development stages. Finally, read outs needed to be suitable for the instrumentation demanded for high-throughput screening for drug discovery. More scalable assays will allow characterization of increased numbers of control and patient neurons.

Conclusion

The iPSC strategy is a novel and complementary approach to model neurodevelopmental diseases. Although this technology is still in its early stage, it

potentially demonstrated the ability to recapitulate relevant neuronal defects of those diseases. This model has the capacity to unify data generated from brain imaging, animal work, and genetics, generating downstream hypotheses that could be tested in well-controlled experiments in the relevant cell types. As several neurodevelopmental disorders share similar neurological symptoms while arising from distinct genetic variations, morphological and functional comparison of patient-specific iPSC-derived neurons would provide insight into common trends and unique phenotypes of each disease. This patient-gene-phenotype cellular analysis can ultimately contribute to the establishment of the links between genes and behavior. In the future, the iPSC approach may also be used as diagnostic tools. Finally, as the technology evolves, it will reach the point of personalized medicine, making predictions about the efficiency of certain drugs and doses in determined individuals.

Acknowledgements

The work was supported by grants from the California Institute for Regenerative Medicine (CIRM) TR2-01814, the National Institutes of Health through the NIH Director's New Innovator Award Program, 1-DP2-OD006495-01, 1R21MH093954 from NIMH, P01 HD33113 and NS22343 from NIH, the Royal Thai Government Scholarship to T.C., the NIH predoctoral training grant T32 GM008666 to A.A and the Emerald Foundation. We would like to thank members of the Muotri laboratory for critical comments on the manuscript. This is a brief review on the fast growing field of disease modeling, we apologize for the omission of important work from colleagues that could not be described or cited here.

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