

Human iPSC Models: A Platform for Investigating Neurodevelopmental Diseases

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Rec date: Jul 09, 2014, Acc date: Aug 08, 2014, Pub date: Oct 20, 2014

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Abstract

Many human diseases arise as the result of DNA mutations in the patient's genome. The neurodevelopmental diseases of early childhood have proven difficult to model due to lack of access to embryonic tissue and ethical concerns. Federal restrictions on the use of embryonic material also preclude studying some stages of neurodevelopmental disease. The onset of illness in utero or early childhood is frequently preceded by normal development of critical milestones. Recent work has led to methodologies to transform somatic cells to embryonic-like stem cells using four exogenous transcription factors. With this approach, it is now possible to validate the use of human induced pluripotent stem cells (hiPSCs) to model aspects of neurodevelopmental diseases using a patient's donated cells or genome editing of hiPSC cells to contain known disease mutations. The reprogramming of somatic cells to hiPSC requires dedifferentiation and resetting of epigenetic signatures in the genome. The newest approaches are evaluating propagating the cells in three dimensions on artificial matrices to recapitulate regional neural cyto-architecture within the brain. Newer genome editing techniques that rely on site-specific sequence recognition by synthetic enzymes can be used to generate hiPSC neurodevelopmental disease models. A hiPSC disease model has several advantages, the patient's own cells may be transduced to provide the investigative cell model and compared to other patient's cells with the same disease. Additionally, a hiPSC model addresses some of the concerns about gene engineered animal models accurately recapitulating human disease since the model context is a patient-specific human cell line. Here we review the emerging use of hiPSC to model neurodevelopmental diseases.

Keywords: Neurodevelopment disease; Embryonic tissue; DNA; hiPSC

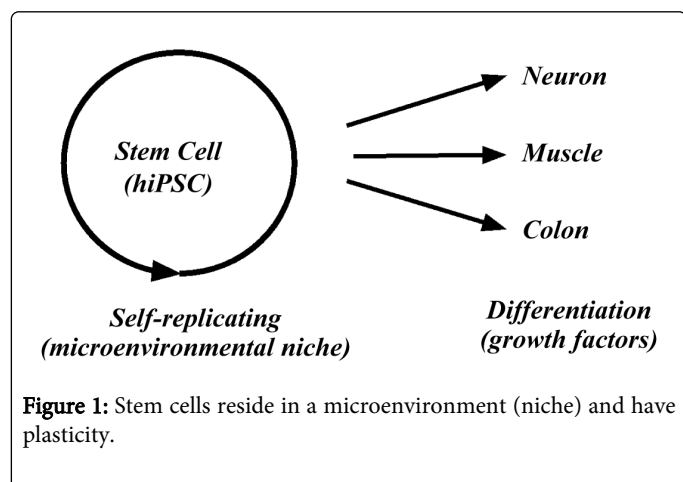
Introduction

Understanding the molecular basis of early developmental disease is hindered by the lack of appropriate human cell and organ models that accurately define disease pathology and progression. Genetically engineered animal disease models have contributed to our understanding of human pathophysiology but questions remain concerning whether small animal models can accurately reflect the human condition. Human cell lines derived from patients with disease are frequently difficult to establish and maintain in long-term cultures. Recently, Yamanaka and colleagues demonstrated that it requires only four introduced transcription factors (Sox2, Klf4, c-Myc, Oct3/4) to reprogram adult somatic cells to dedifferentiated induced pluripotent (hiPSC) cells [1-5]. With this innovative methodology, many laboratories are using hiPSC to model human diseases that occur in many organ systems [6-14]. While hiPSC are a promising investigative method to define molecular deficits in disease cells, it will require rigorous comparisons between many patients with the same disease to clearly characterize cellular dysfunction and abnormalities [15]. The ability to produce hiPSC models of human disease represents an opportunity to capture disease pathophysiology during disease progression. Here we summarize some of the emerging hiPSC cell themes and review the use of hiPSC methodology to study human neurodevelopment disorders.

Generation and Epigenetics of hiPSC cells

The Yamanaka method of reprogramming cells to a pluripotent state has been modified with many improvements but is not without limitations. The process of generating induced pluripotent stem cells (hiPSC) from somatic cells by exogenous transcription factors is made possible by epigenetic changes that take place during the reprogramming process (Figure 1) [3,16-19]. The derivation of fully reprogrammed hiPSC is achieved through establishment of embryonic stem cell-like epigenetic changes permitting the reactivation of key endogenous pluripotency-related genes, establishment of appropriate bivalent chromatin domains and DNA hypomethylation of genomic heterochromatic regions [17,20,21]. Reconstitution of the epigenetic genome is a very inefficient process and the vast majority of the induced cells fail to complete the reprogramming process. Researchers are able to manipulate somatic cells (primarily fibroblasts from skin and PMBC from blood) and reprogram them to hiPSCs by ectopic expression of the pluripotent transcription factors Oct4, Sox2, Klf4, and c-Myc (or Nanog and Lin28 instead of Klf4 and c-Myc) [22-25]. To increase efficiency, accelerate kinetics, and reduce safety concerns, many improvements in methodology have been achieved. Several cell types are now shown to be reprogrammable with high efficiency. The newer methods involve overexpression of exogenous hiPSC transcription factors, manipulations involving the administration of cytokines or small molecules generating stem cells more rapidly and with somewhat higher efficiencies. Methods using virus-free, removable transposons, adenovirus, mini-circle systems, or episomal systems have been developed [26-30]. Although generating hiPSCs by these means are successful, the use of exogenous DNA constructs leaves the possibility of genomic integration into the patient cells. Other methods generate hiPSCs by using non-integrating Sendai

virus-mediated gene delivery to reprogram hiPSCs, but their use may be limited due to cost [31,32].

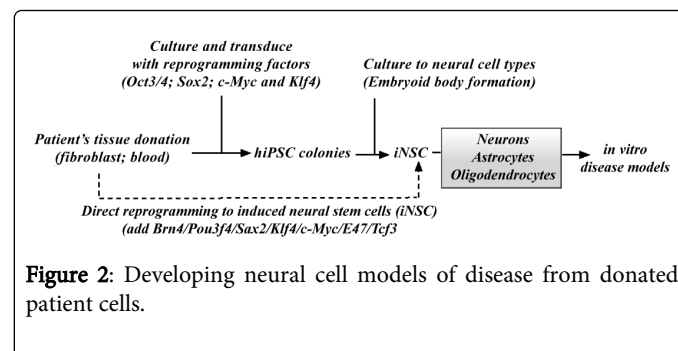


Using growth cocktails they can be re-differentiated to many cell types to model cell function. These techniques allow a scalable source of cell material for *in vitro* study.

There are now promising strategies to regenerate neural progenitor and specific neural lineages [33,34]. It is possible to cause the trans-differentiation of mature fibroblasts directly into induced neurons again by forced expression of key transcription factors, skipping the intermediate pluripotency stage (Figure 2) [35]. Trans-differentiation to neural cell types has the advantage of avoiding making the intermediate hiPSCs stage to derive patient-specific cells. This approach makes the process faster and more efficient and may reduce the risk of pluripotency-associated tumorigenesis and possibly avoids immunogenicity if these induced neural cells (iNCs) were to be used for gene therapy [36]. These induced neurons can form synapses and generate action potentials. In this approach, the source tissue has a finite capacity to replicate. However, trans-differentiation may skip a developmental window during which a molecular deficit underlying disease is manifest. Despite these concerns, the iNC cells have the properties of self-renewal and have developmental plasticity, making them a promising resource for modeling neurodevelopmental pathogenesis, drug screening, and cell based therapy [37,38].

Epigenetics studies the functionally relevant alterations in genome activities that do not result from DNA sequence changes [20,39]. Epigenetic changes are represented by DNA (and RNA) methylation patterns and histone modifications of the chromatin [16]. Epigenetic activity is seen in the cellular differentiation process going from totipotent cells in a zygote to define cell types (liver, muscle or neuron) as the developmental program is completed. The reprogramming of somatic cells (fibroblasts and peripheral blood mononuclear cells) to hiPSCs by introducing exogenous transcription factors is made possible by epigenetic changes. Completely reprogrammed hiPSCs re-acquire activation of key endogenous pluripotency-related genes [40]. Reported epigenetic changes in remodeled hiPSC include appropriate epigenetic bivalent chromatin domains, DNA hypomethylation of heterochromatic regions and satellite repeats, reactivation of inactive X chromosomes in female hiPSC, and resetting of imprinted loci to the embryonic signature [41]. However, resetting the pluripotent epigenetic signature in somatic cells is a very rare event (estimated at <0.1% of differentiated cells exposed to reprogramming factors [42]. Two classes of cells are obtained during reprogramming: early/

intermediate, pre-hiPSC (partially reprogrammed) and completely reprogrammed hiPSC cells. It is suggested that pre-hiPSC may progress further to fully reprogrammed hiPSC with extended culture time. As the molecular epigenetic chromatin signature is reprogrammed in hiPSC, three-dimensional reorganization of chromatin structures and nuclear subdomains is acquired [19]. Efficient epigenetic reorganization is a requirement for reliable downstream *in vitro* modeling of patient-specific diseases and clinical therapeutic uses of hiPSCs.



Patient cells are used to develop induced pluripotent stem (iPS) cells that can be induced to form neural cell lineages or trans-differentiated directly to neural stem cells (iNSC) that can form the neural lineages .

Microenvironment, Three Dimensional Scaffolds and Organoids

The physical location and chemical microenvironment of stem cells regulates whether the stem cell will retain its plasticity (self-renewal) or will enter a differentiation pathway [43-45]. The intimate balance between these two stem cell fates regulates homeostasis and tissue repair [46,47].

Most multi-cellular tissues are composed of at least two cell types. Understanding the complexity of cell-cell and cell-extra cellular matrix interactions is needed to build better model systems to study neurodevelopmental processes [48]. The development of three dimensional (3D) hiPSC cultures relies on having a biocompatible material to seed the cells on. This configuration encourages cell attachment and proliferation with hiPSC producing their own extracellular matrix (ECM). It also provides structural stability to maintain the trans-differentiated cell phenotype [49]. Available 3D matrices are limited and not always homogeneous. Popular substrates are based on collagen or cellulose hydrogels [50]. Matrigel, isolated from mouse tumor tissue, is expensive yet appears to provide a good ECM alternative. The expectation is that the cells will self-organize once introduced into the 3D matrix. The 3D organization of stem cells permits interactions that resemble embryogenic processes including morphogenesis, adhesion, biomechanical and biomolecular communication between cells [43,51].

Three dimensional (3D) cultures may have advantages over the standard two dimensional cell cultures. The 3D cultures appear to capture more information on cell behavior, such as cell-cell interactions and cell polarity orientations that may impact gene activity [52]. 3D systems may better model the cell's microenvironment by more closely replicating the physiological tissue environment [47,53]. In these systems, it is important to show functionality. A recent study by Munhall, et al. [54] compared

epithelial cancer cells grown in monolayer culture (2D) and in a 3D system. The electrophysiological extracellular conductivity in 3D grown cells was higher than 2D grown cells. This group also reported differences in cell morphology and intracellular trafficking. In another study, Itoh, et al. [55] reports the development of a 3D culture of fibroblasts and keratinocytes from hiPSC to model skin dynamics. This 3D system may be useful for defining the etiology of skin diseases [55]. Both sets of findings argue that 3D cell models can be developed to better mimic the *in vivo* tissue environment. Yet, 3D organoid cultures are limited by length of preparation time (months), appropriate ECM substitutes, the equipment to set-up and expensive consumables. However, investigators are now developing scaffolds to facilitate the derivation of 3D cultures especially for neuronal investigations [6,56]. Many cellular assays will need optimization and/or validation for 3D cultures prior to *in vitro* comparisons [57]. Finally, there are not yet enough data to show duplication of findings between laboratories.

The complexity of the human brain makes it difficult to study brain disorders in model organisms [34,58,59]. There is a need for *in vitro* models of human brain development. A promising approach now being developed in hiPSC research is the three-dimensional organoid culture [60,61]. Recently, human hiPSC were coaxed to generate a three-dimensional neural cell structure, termed a cerebral organoid, that developed various discrete, interdependent and multiple layered brain regions [62,63]. Interestingly, cerebral organoids recapitulate features of human cortical development, including the characteristic progenitor zone organization with abundant outer radial glial stem cells [63]. Three-dimensional neural organoids grown from hiPSCs hold promise for elucidating phenotypes of neurodevelopmental disease since these types of cultures more closely define the cyto-architecture and the complexity of the neural circuitry in the brain.

hiPSC Models as Platforms to Study Neurodevelopmental Diseases

Neurodevelopmental disorders, like autism spectrum disorders and X-linked intellectual disability disorders are a group of syndromes of unknown etiology characterized by deficits in language, development of personal communication, reciprocal social interactions and restrictive behavior [35,64-66]. There are few pharmacological treatments for these syndromes or even identified targets for drug development. During the last four decades, a large number of mutations on the X chromosome have been documented as contributors to intellectual disability syndromes including Fragile X (FMR1 loci), Rhett (MECP2 loci) and Lesch-Nyhan (HGPRT loci) (reviewed in [67,68]). The preponderance of cognitive disability causing mutations on the X chromosome has raised the possibility that the X chromosome is a repository for many genes regulating cognitive function and/or brain development [69]. Mutations in genes (ex: SCN1A, UBE3B and TBX1) associated with decreased cognitive ability also map to autosomal locations. Consequently, the availability of *in vitro* model systems for these diseases would allow a better understanding of the development of functional defects arising from known genetic defects [70,71].

Neurodevelopmental studies in animal models [72] that mimic human cognitive developmental processes may be limited by the ability to assess cognitive function in these models. The BTBR mouse exhibits neurobehavioral deficits and some diagnostic symptomology for autism and has become a model for pharmacological testing [73-75]. Disruption of SHANK3 in the mouse leads to behavioral and developmental deficits resulting in autism spectrum-like phenotypes.

Mouse models of other SHANK protein family members are providing insight for neuronal synaptic and postsynaptic density aberrations that may affect cognitive function [76]. Mouse models also exist for other neurodevelopmental syndromes such as Downs [77-79], Fragile X [80] and Rett Syndrome [81]. However, direct human tissue assays are desirable to investigate these disorders. Availability of tissues or biopsies from the critical early developmental period in patients rarely can be obtained due to ethical concerns and access limitations. Consequently, hiPSC lines (directly or indirectly differentiated to neuronal cell types) may offer the best approach to defining the role of putative gene mutations during the neurodevelopmental period. They provide a novel patient-derived resource to experimentally define human neurodevelopmental disease.

The neurodevelopmental cognitive dysfunction syndromes represent a genetically heterogeneous disease grouping with complex neuropathology that may intersect in common pathways that result in neural activity deficits [82,83]. One of the most advantageous aspects of hiPSC models is that the patient's cells become the source of the disease model [11]. These cells contain the genetic mutation in the context of the patient's genome including other potential risk factors such as modifier genes. The introduction of hiPSC methods to investigate neuronal activity and function in the developing cyto-architecture of the brain, the intrinsic wiring of the synapses, neuron metabolism and energy requirements may provide clues to understanding how cognition is acquired. Several groups show that hiPSCs can be directly or indirectly differentiated to neuronal cell types. Induced pluripotent cells have the capacity to develop into neural stem cells (iNSC) and, when cultured with specific growth cocktails, to support the outgrowth of the neural subtypes (such as neurons, astrocytes and oligodendrocytes [64,84-86]). The development of the neuronal cell types can be ascertained by screening for known biomarkers (e.g., NSE, GFAP). Thus, patient derived hiPSC cells may be used to create early neurodevelopmental pathology models for study.

Several groups have generated disease-specific lines from patients with monogenic autism spectrum disorders including Rhett [87], Fragile X [88] and Timothy Syndromes [89]. The objectives of these studies are to identify functional disease-specific phenotypes and to examine whether these phenotypes can be rescued by therapeutic interventions. Another objective is to use patient hiPSC models to understand the cellular and molecular changes that occur in these diseases. Several studies have looked at gene profiling of disease hiPSC compared to human donor tissue obtained from brain autopsies or control hiPSC lines [90-93].

Fragile X Syndrome (FXS), the most common of the Autism Spectrum Disorders, results from a trinucleotide repeat in the FMR1 gene and a subsequent loss of FMRP protein [94,95]. FMRP functions as an RNA binding protein where it regulates aspects of translation, stability and cell localization. A lack of FMRP enhances intracellular signaling in the mTOR, GluR5, ERK, GSK3 β , insulin and PI3K pathways [88]. Neural stem cell plasticity and this cell's ability to change phenotype responding to signals from the environment may be impacted by the FMRP protein [96]. Human iPSC were obtained from fibroblasts obtained from three well-characterized Fragile X patients. The patient's FMR1 mutation was maintained in the hiPSCs. Forebrain neurons differentiated from the hiPSCs showed defective neurogenesis, presynaptic dysfunction [94] and decreased neurite length [97-99].

Another study using hiPSC created from FXS patient cells showed that the FMR locus was resistant to histone methylation; the functional significance remains unknown [100]. These studies potentially begin to link FMR protein dysfunction with developmental cognitive phenotype.

Rhett Syndrome, an X-linked neurodevelopmental disorder, primarily affects females and is usually lethal in hemizygous X males. Rhett patients have postnatal microcephaly, speech and developmental milestone losses, growth retardation and autistic behaviors [101,102]. Estimates suggest that a large majority of the Rhett Syndrome cases arise from *de novo* mutations in the methyl CpG binding protein 2 (MECP2) genes. Mouse knockout models demonstrated that MECP2 is critical for neuron function [87]. MECP2 function is located in several neuronal cell types including inhibitory and excitatory forebrain neurons. Several groups have functionally characterized MECP2 deficient neurons derived from patient hiPSCs [87,103-105] and reported reduced cell size, lowered expression of neuronal biomarkers, decreased transient intracellular calcium level, decreased response to excitatory and inhibitory stimuli and fewer action potentials. Thus, neural cells derived from patient hiPSC are beginning to document that specific neuronal deficits of MECP2 protein have global functional impacts in Rhett Syndrome [103].

Timothy Syndrome is a channelopathy caused by a point mutation in the $\alpha 1$ subunit of the L-type calcium channel ($Ca_v1.2$) resulting in inactivation of the channel. These patients have autism, cardiac dysfunction (long QT) and other developmental delay phenotypes [89,106,107]. Well-defined cortical neurons generated from patient hiPSC display calcium signaling deficits and variations in related gene activities. These cells also show changes in expression of tyrosine hydroxylase and overproduce norepinephrine and dopamine. Electrophysiological analyses of control neurons showed no differences in action potential generation, resting potential or action potential amplitude. In contrast, the Timothy Syndrome induced neural cells showed alterations in action potentials that were wider, pointing to a loss of channel inactivation. This study suggests that Timothy Syndrome patients have defects in action potential firing and calcium signaling. Another study generated hiPSC to address cardiac function in Timothy Syndrome patients; these findings suggest deficits in cardiomyocyte contractile ability, electrophysiological signaling between cardiomyocytes and calcium signaling [107]. Gene expression arrays showed gene profiles in Timothy Syndrome derived cortical neurons differed from control cells. The Timothy Syndrome gene profile showed activity distortions in 11 genes previously implicated with other autism spectrum or intellectual disability disorders suggesting, perhaps, a cellular deficit associated with a common cellular intersection during neurodevelopment.

Dravet Syndrome, another channelopathy, has also been studied using a hiPSC model. Infantile-onset epilepsy syndrome is a debilitating neurodevelopmental disease that has cognitive and autistic features [108] resulting from *de novo* mutations in the SCN1A gene (chromosome 2). It develops in a previously normal infant during the first year and the epileptic seizures are refractory to current drug treatments. Psychomotor development slows by the second year and patients show other declines in mental development; 10-20% of the patients die from the disease. The SCN1A gene encodes the α -subunit of the voltage-gated sodium channel $Na_v1.1$. In 50% of the patients, the protein is shortened prematurely during translation. Knockout-knockin mouse models suggest $Na_v1.1$ channel haplo-insufficiency accounts for the pathology where action potential generation is

impaired in rodent GABAergic forebrain neurons [109]. Autistic behavior in heterozygote SCN1A mice is also linked to defective GABA neurotransmission [110]. Hirgurashi et al., [108] approached the syndrome's pathology by using hiPSC to model and to evaluate cell impairment in Dravet patients. Patient hiPSC were made from fibroblasts identified to have a point mutation (c.4933C>T) that resulted in a truncated $Na_v1.1$ channel protein (all other closely related Na^+ channel genes in the patient were wildtype). Neural cells differentiated from hiPSC neurospheres expressed neuron (tubulin III) and astrocyte (GFAP) markers. Electrophysiological studies, comparing Dravet and control derived hiPSC neural cell responses, demonstrated that the Dravet neurons (largely GABAergic neurons) malfunction during action potential generation using sustained current. This study shows the potential to dissect and understand how human GABAergic neurons contribute to Dravet pathophysiology although several more patient hiPSC models are needed to validate these findings as a common result.

Other studies have modeled imprinting disabilities like Prader-Willi and Angelman Syndromes [9] in hiPSC lines. A common theme of the described studies is the use of hiPSC models derived from patient material to address the pathophysiology of neurons within the first years of the patient's life. These studies utilize patient neuronal cells derived from hiPSC; these cell types are not easily obtained by other means and represent a novel approach to solving how neurodevelopmental dysfunction may impact these syndromes.

Creating Human Disease Models in hiPSC by Genome Editing

Historically, causal relationships between disease and gene function have been studied in cell or animal models by engineering gain or loss of function mutations into the genome. The introduction of gene knockouts or "humanized" knockins by gene replacement in mice to model disease is a proven approach [111-114]. However, the complexity of three-dimensional anatomical connections and the physiology of species-specific metabolomes are different and may lead to erroneous conclusions when modeling human disease in other organisms. Human tissues for study may be acquired at autopsy but postmortem tissues represent a fixed endpoint of disease. The ability to produce hiPSC models of human disease represents an opportunity to capture and/or recapitulate disease pathophysiology in human cell model. Utilizing patient cells with inherited mutations or hiPSC engineered by genome editing to contain common disease mutations provides a unique approach and may overcome some of these criticisms. The hiPSC technology is an alternative to animal models providing a human tissue surrogate for research that is scalable and sustainable.

Genome editing is an emerging approach to model disease based on the ability to introduce either gene knockouts or patient-specific point mutations into a hiPSC genome [115-117]. Sequence specific DNA recognition is the hallmark of the synthetic editing nucleases described here. The methods rely on introducing a DNA double strand break initiated by a pair of DNA binding-nuclease protein modules. These bind a unique DNA sequence creating specificity for the dsDNA break. Methods initially employed were adapted using Zinc Finger Nucleases (ZFN), a DNA binding protein linked in modules to recognize a unique DNA sequence. The ZFN module is also linked to a DNA restriction endonuclease (Fok I) that cleaves genomic DNA. Introduction of the engineered ZFN modules into the cell produces a dsDNA strand break left and right of the selected DNA target and

activates endogenous DNA repair mechanisms in the cells that may incorporate deletions or insertions. This activity results in different size gaps in the genome (knockout) or using an exogenously supplied template incorporates mutations into the genome (similar to the homologous replacement vectors used in mouse genome engineering). One drawback of this approach is the difficulty in optimizing the ZFN modular protein recognition sites needed for DNA cleavage. ZFN pairs also show context dependence making it difficult to estimate each module's binding specificity. These two problems make "off-target" outcomes more likely when using ZFN technology. Hence, ZFN pairs require extensive *in vitro* testing making them an expensive choice for genome editing. ZFN genome editing is more fully reviewed in [118,119].

More recently, TALENS (transcription activator-like effector nucleases) systems were utilized to introduce mutations into hiPSC genomes. TALENs are derived from a bacterial plant pathogen that facilitates plant infections using TALE proteins [120]. The DNA target sequence needed for the left and right side TALE protein is 16 nucleotides and they are very selective. These recognition modules also are linked to the Fok I endonuclease. The TALE cutting of DNA is efficient and their design may restrict "off-target" effects making this approach more generally usable compared to ZFNs. However, there are some requirements that make TALENs problematic. Their binding is sensitive to 5-methylcytosines in the DNA and there is a requirement for a T base to precede the recognition site. However, in the short time that TALENs have been used for genome editing, a TALEN library is available that predictably targets over 18,000 genomic sites [121] making the TALEN approach economically and strategically easier. TALEN technology is further reviewed in [122,123].

Another genome editing approach, the CRISPR/Cas9 system, adapted from a bacterial immune system and reported in 2013, employs a single strand RNA guide (sgRNA) to direct the Cas9 nuclease to the targeted genomic locus [124]. For genome editing, the sgRNA is specific for the targeted genomic site and is co-introduced into the cell with the Cas9 nuclease. The CRISPR/Cas9 system appears the most useable by all laboratories since it requires less complex modules that act on DNA and the sgRNA can be synthesized for editing/targeting specific endogenous DNA sequences. This technology is reliable and highly efficient in mice [125] and is being utilized in hiPSC disease modeling [126]. Yet, there are some use constraints. Cas9 can produce "off-target" cleavage because it is permissive for mismatches in the sgRNA. Consequently, as with all the described genome editing methods, hiPSC genome screens should be conducted to confirm the introduced deletion, insertion or mutation. The CRISPR/Cas9 approach is rapidly becoming the genome editing technology of choice and is further reviewed in [127,128].

One of the promises of regenerative medicine is to rescue the patient from disease by *in vitro* correcting stem cells *ex vivo* with genome editing and transplanting the corrected cells back to the patient [129,130]. This has been done successfully with hemopoietic stem cells [131,132] for a few diseases and is now being investigated for a number of conditions [133-137]. The potential to explore hiPSC models of neurodevelopmental disease using genome editing techniques is a promising and exciting area just beginning to emerge from hiPSC technology (Figure 3).

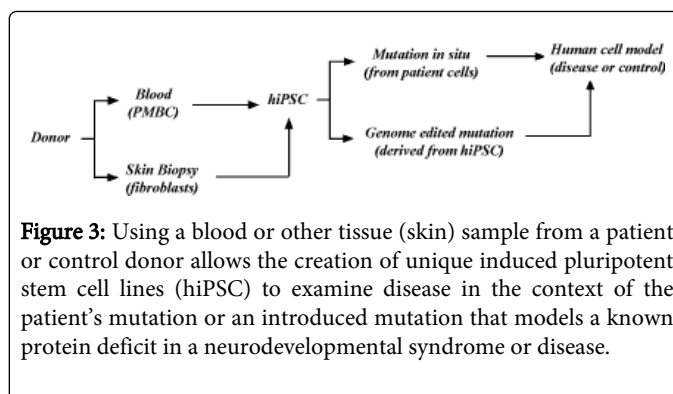


Figure 3: Using a blood or other tissue (skin) sample from a patient or control donor allows the creation of unique induced pluripotent stem cell lines (hiPSC) to examine disease in the context of the patient's mutation or an introduced mutation that models a known protein deficit in a neurodevelopmental syndrome or disease.

Limitations of hiPSC Models

Induced pluripotent stem cells hold promise for future research paradigms and work in progress will refine our approaches and define safe and efficient protocols for derivation of hiPSC models. However, there are current limitations to hiPSC use. For the hiPSC approach to be used as a neurodevelopmental model, researchers need to validate that the derived hiPSC stably expresses the disease phenotype. Also, if using the direct reprogramming approach (somatic cell→neural cell), there are a limited number of mitotic cell divisions that occur and it may take long-term culturing of the cells to obtain neural connectivity. Some vectors (retroviral and lentivirus) used to reprogram hiPSCs persist in the cell's genome as foreign pro-viral sequences. Using these cells as regenerative tissue sources or for gene therapy protocols raises biosafety concerns. Some hiPSC are reported to exhibit high phenotypic variability. Reports of genetic (chromosome aberrations; common trisomies reported for chromosomes 12 and 17 similar to those seen in mouse ES cells for chromosome 8) and epigenetic instability even among clones derived from the same cell source may be problematic [138,139]. Hence, the hiPSC lines must be rigorously characterized, including karyotype, before use as a disease model or before assaying *in vivo* therapeutic responses. When developing transdifferentiation protocols to generate neuronal cell lines relevant to neurodevelopmental diseases, the choice of cell type, regional brain specificity, recapitulation of a neurodevelopmental program, and functional connectivity (synaptogenesis) should be evaluated. Furthermore, undifferentiated hiPSCs co-cultured with mouse feeder cells or undefined media constituents may introduce "noise" into the phenotypic characterization. As discussed earlier, the epigenetic reprogramming process is inefficient and many hiPSC fail to complete the process. Heterogeneous neuronal cultures can acquire variability when generated from various types of neurons and from differing states of functional maturation. Awareness of these challenges should be considered when planning any *in vitro* cellular phenotyping of hiPSC disease models. Today, many studies are focused on refining culture conditions with controlled supplements and 3D culture platforms that mimic the microenvironment and that recapitulate the cyto-architecture of neurodevelopment.

Conclusion and Future Directions

hiPSC represent an important and innovative tool to model and to investigate neurodevelopmental disorders [140]. They are able to recapitulate a developmental program and can be directed to become a selected neural cell type. Consequently, researchers can approach and

analyze disease specific mutations by differentiating hiPSCs to the desired neural cell types and/or build neural organoids [34]. These manipulations potentially target early embryonic developmental programs not addressable by other methodologies. Reprogramming patient somatic cells to hiPSC eliminates some of the ethical issues with embryo-derived cells and allows the isolation of patient specific mutations for study.

The hiPSC technology holds great promise for patient-specific stem cell based therapies and the production of *in vitro* models of human disease, the so-called “disease in a dish” models. This technology also provides an opportunity to perform experiments on human cells that were not previously possible, such as high through put drug or chemical screens to identify compounds that may inhibit or reverse disease progression [104]. In the future, hiPSC may provide scalable and sustainable tissue sources for regenerative medicine as shown with gene corrected hemopoietic stem cells [43,141,142] used for bone marrow transplant studies to alleviate disease. hiPSC retain the genetic constitution of the patient and due to the cell’s plasticity provide a good model for linking cell functionality in neurodevelopment with disease phenotype. Due to safety issues and requirements for more advanced regenerative technology the current utility of hiPSCs is *in vitro* disease modeling from patient donor cells. The use of hiPSC to create and study neurodevelopmental disease platforms is an exciting advance and holds promise for changing our approach to these diseases [143,144].

Acknowledgments

The authors wish to thank Dr. Cooduvalli Shashikant of the Penn State University Genome and Bioinformatics Program for a critical reading of the manuscript. The authors state that they have no conflicting interests.

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