

Patient heal thyself: modeling and treating neurological disorders using patient-derived stem cells

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Abstract

Disorders of the brain and spinal cord are common worldwide problems but have remained very difficult to treat. As a group they have diverse etiologies and can be due to trauma, infection, tumors, genetic mutations and environmental insults. Though distinct in etiology, neurological disorders share an overall intractability as current therapies are largely limited to treatment of symptoms. Improved outcomes are further constrained by the minimal endogenous capacity of the brain and spinal cord for repair. Spectacular recent scientific advances, however, suggest that new stem cell-based approaches may change this undesirable situation. In this review, I will broadly outline the challenges of studying and treating disorders of the brain and spinal cord. I will review ongoing attempts to use stem cell-based therapies to both model and treat neurological disorders. While this field is in its infancy, expected advances and needed breakthroughs point to a future where patient-derived stem cells will be the basis for the emerging discipline of regenerative neurology.

Keywords: induced pluripotent stem cell, iPSC, mesenchymal stem cell, neural regeneration

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Introduction

Disorders of the central nervous system (CNS) are due to highly diverse etiologies that include genetic, traumatic, vascular, infectious and environmental causes. While varied in cause, they unfortunately share a high prevalence, enormous cost to society worldwide and in general, a generally poor response to existing treatments. Current medical and surgical therapies are largely restricted to alleviation of symptoms though emerging approaches (for example, 'neuroprotection' in epilepsy) aim to arrest disease progression in mildly affected or presymptomatic individuals. These include the large numbers of people at risk for Parkinson's or Alzheimer's disease and the current lack of any truly effective therapies.^{1,2} Despite recent progress, there exists a tremendous need for novel therapies that can not only reverse neurological deficits but either enhance the minimal endogenous capacity for neural regeneration or exogenously provide repair. A profoundly different approach to treat neurological disorders will likely be required to achieve an all-elusive 'cure'. Such musings were once restricted to medical fantasy or science fiction for many years but can now be discussed in a more meaningful and rigorous way. This is certainly attributable to many innovations in science and medicine that have been realized over the past 100 years. However, over the last

few years we have seen dramatic progress, spurred by spectacular advances in stem cell biology. Most notably, patient-derived stem cells and their potential use for disease modeling and therapeutics should fundamentally change how we approach and treat neurological diseases.

Though all fields of medicine will likely utilize patient-derived stem cells, their application to the fields of neurology and neurosurgery should be especially meaningful given the stark limitations of current therapies. While there is an increasing appreciation of brain plasticity, the restricted regeneration of the human brain and spinal cord seems to be a major obstacle towards significant treatment success. In this review, I will discuss the application of patient-derived human stem cells to both model and treat neurological disorders. I will also speculate on likely directions of basic and translational research over the next several decades. Such contemporary and future breakthroughs are expected to lead to a new clinical reality where patient-derived stem cells can be generated, expanded, modified and ultimately given back to patients to treat neurological disorders.

Stem and progenitor cells

Stem cells have been extensively reviewed and have been the subject of much scientific interest.^{3–7} In addition, they

have clearly captivated public attention, speculation and controversy over methods of derivation and appropriate usages.^{8,9} Here, I define 'stem cell' as having the capacity to replicate indefinitely while maintaining an ability to form cells from the ectoderm, endoderm and mesoderm lineages. The best studied examples to date of course, are embryonic stem cells (ES)^{10–12} as well as induced pluripotent stem cells (iPSCs, see below). In contrast to stem cells, progenitor cells are limited in their replicative potential and differentiation status, typically only able to give rise to restricted cell types or single lineages. Terminology used in the primary scientific literature and the lay press is unfortunately inconsistent and confusing as progenitor cells are often also termed 'stem cells'. Salient examples include hematopoietic progenitor cells usually employed for bone marrow transplantation and mesenchymal 'stem' cells (MSC) derived from adipose tissues, bone marrow or umbilical cord blood. Neural progenitor cells (NPC) are also a highly relevant example of cell-based therapies that already have been employed as possible regenerative therapies for neurological diseases.^{13,14} These progenitor cells are again defined by limited self-renewal with a restricted capacity to give rise to neurons and glia. However, their inherent propensity to migrate within a host brain makes their potential application for transplant and regenerative therapies particularly noteworthy. As done in the past for the treatment of Parkinson's disease, NPC can be harvested from human fetal sources and then directly given to patients.¹³ However, multiple problems exist including ethical concerns due to the fetal source of NPC, medical complications such as immunorejection of transplanted cells and lack of clear evidence for efficacy. The future use of NPC are more likely then to be generated *in vitro* from autologous stem cells such as iPSC. This approach should also allow more control over the specific type of human neural progenitor and derivative cells that can be formed and ultimately applied as a regenerative therapy for neurological diseases.

Induced pluripotent stem cells

Yamanaka and Takahashi¹⁵ first reported iPSCs derived from mouse fibroblasts in 2006 in an extraordinary and comprehensive paper published in *Cell*. A similar feat generating iPSC using fibroblasts derived from adult humans quickly followed the next year, reported again by Shinya Yamanaka's laboratory as well as that of Jamie Thompson.^{16,17} Their landmark work was quickly reproduced and expanded by many other laboratories. The field of iPSC biology then underwent an initial period of excitement and frenzy with many publications seemingly limited to reporting minor variations in methods of reprogramming as well as defining the minimal set of exogenous genes required to re-program somatic cells. As this field developed and matured, additional emphasis was placed on mechanisms of re-programming as well as differences and similarities to ES cells and early examples of human disease modeling using iPSC.^{18–23} This nascent field and the rapidly expanding body of knowledge over the last

five years quickly culminated in the Nobel Prize (for Physiology or Medicine) being awarded to Shinya Yamanaka and John Gurdon in 2012.

While critically important, this is certainly just the first chapter of this story, with much more knowledge to come with focused uses for science and medicine. Indeed, the seminal advances based on iPSC now permit rational speculation about regenerative medicine including application to neurological disorders. The future may then feature treatment paradigms where somatic cells from individual patients will routinely be re-programmed to iPSC, modified *in vitro* as necessary and then given back to patients. A 'blueprint' of this pathway and expected challenges is outlined in more detail below. This overall approach is probably the purest example of 'personalized medicine' as one's own cells can directly employed as therapies for neurological as well as other disorders.^{24–26}

With all the excitement and hype of the last few years, it is worthwhile to ask: why is there such emphasis on human iPSC? While it is tempting to jump to their application as regenerative therapies (see title of this review!), patient-derived stem cells such as iPSC are already providing highly relevant information as *in vitro* disease models. They can be used to generate human neurons *in vitro* and used to ask specific questions about aberrant neuronal differentiation and function that may underlie disorders such as epilepsy, autism and intellectual disabilities. They can also be employed for assays of neuronal function using electrophysiology. In addition, patient-derived iPSC and derivative cells such as neurons can be employed for higher throughput drug screening. This may culminate in the identification of novel treatments that work for human disease. The last point is notable as there have been many examples of 'therapies' that are quite effective in mouse and other animal models that did not translate well to human patients.²⁷ Finally, human neural cells derived from patient iPSC may be more capable of modeling critical aspects of complex neurological and psychiatric diseases such as schizophrenia, bipolar disorder and personality disorders. These often disabling conditions will be very difficult to study using rodent models due to evolutionary differences between mice and humans and the likely complex genetics that underlie these disorders. The application of iPSC-derived neurons to model human neurological and psychiatric disorders was recently succinctly reviewed by Dolmetsch and Geschwind.²⁸

Mesenchymal stem cells

MSC have been extensively studied and have a potential role in regenerative medicine given their intrinsic properties and the relative ease of procurement from patients. However, I will not extensively discuss MSC in relation to modeling and treatment of neurological disease, as they have recently been reviewed²⁹ and their usage is projected to be increasingly overshadowed by iPSCs. Furthermore as defined above, MSC are better classified as progenitor, not stem cells.

The most common application to date of MSC for neurological disease is to treat multiple sclerosis, a demyelinating autoimmune disorder.^{30–32} When transplanted into animal models of multiple sclerosis or human patients, MSC appear to have some capacity to enhance neural repair though it is currently unclear if this is due to cell autonomous effects (transplanted cells themselves integrating to the host nervous system and improving function) or non-cell autonomous mechanisms (transplanted cells not directly participating in repair but rather modifying endogenous factors or pathways such as inflammation that may lead to improved CNS function).^{30,33} While groups have shown a capability to form neuron-like cells from MSC,³⁴ their ultimate ability to function *in vivo* is not established. MSC then may not be able to serve primary roles during neural regeneration.

Disease modeling

As discussed below, there are many barriers to be overcome before patient-derived stem cells are in routine clinical use for regenerative therapies. However, these cells have already been very effectively employed for modeling diseases including amyotrophic lateral sclerosis,¹⁸ Huntington's and Parkinson's diseases³⁵ and many other disorders. Patient-derived iPSC from patients with well-characterized phenotypes and genotypes will be invaluable to increase our knowledge of many neurological disorders. Use of these new cellular tools will allow the generation and testing of novel hypotheses and are expected to increase the pace of discovery.

Future work using human iPSC will closely intersect with developmental neurobiology to advance our knowledge of how specific populations of neurons and glial cells are generated.³⁶ The availability of appropriate 'starting material' of patient-derived stem cells and known cellular deficits (such as spiny medium neurons that are lost in Parkinson's disease) will greatly facilitate experiments and focus on developmental pathways relevant to human disease. Another example is provided by Rett syndrome, a severe neurodevelopmental disorder classically caused by mutations in the *MECP2* gene though some patients with atypical features instead have mutations in the *CDKL5* gene. iPSC have been generated from fibroblasts obtained from patients with *MECP2* and *CDKL5* mutations.^{20,37} *MECP2* mutant iPSC were then differentiated to excitatory glutamatergic neurons, these authors found functional neuronal deficits and reduced numbers of dendritic spines.²⁰ Excitingly, these neurons were used to test therapies that increased production of *MECP2* protein as well as restored numbers of excitatory glutamatergic synapses. This example shows how patient-derived stem cells can effectively complement other experimental approaches focused on the use of animal models.³⁸ The combination of patient-derived stem cells with appropriate animal models may in fact be an ideal approach to quickly identify both efficacious and safe therapies for neurological diseases.

Challenges and obstacles to regenerative therapies with patient-derived stem cells

As suggested by the title of this review, the ultimate application of patient-derived stem cells may be their use as cellular replacement therapies. There are many hurdles to achieving this goal.

First, stem cells need to be generated without the use of exogenous DNA or permanent genomic modification of patient cells. Most re-programming to date has been done using retroviruses or lentiviruses to transduce dermal fibroblasts isolated from patients.³⁹ The random integration of these viruses may inadvertently inactivate or activate endogenous genes based on the stochastic insertion of these viruses. Moreover, these viruses harbor strong promoters and these may be re-activated during iPSC differentiation. Such genetic changes would greatly diminish enthusiasm from ever using these cells as therapies. However, they may still be useful for disease modeling though the genetic changes described above need to be carefully considered when interpreting any phenotypes. Re-programming alternatives to viruses include plasmid-based transient transfection⁴⁰ as well as the introduction of proteins, RNA or small molecules that can re-program target cells.^{41–43} Patient-derived stem cells should ideally never be exposed to any animal products (for example, bovine serum) or animal cells that could transfer retroviruses and potentially compromise safety when re-introduced back to patients. There were early concerns about the quality of iPSC generated from patients if additional genetic mutations were being introduced during re-programming. A recent study found that much of the copy number variation previously noted in various lines of human iPSC was already present in parental fibroblasts and likely reflects the clonal nature of iPSC.⁴⁴

Second, patient-derived stem cells should not retain epigenetic 'memory' from the cell of origin such as a fibroblast. This is a likely a chromatin-based mechanism that may restrict developmental potential and possibly prevent, for example, the production of fully differentiated and functional neurons.^{45,46} Advances in our understanding of re-programming should address this concern but this aspect of stem cell biology will require much more study.

Third, the appropriate delivery of cells to the brain or spinal cord is not at all established. Simple injection of cells into the central nervous system raises multiple points of concern about the proper delivery site, 'dose' of cells and the timing and number of treatments. Furthermore, will transplanted cells need to be delivered with a permissive matrix or growth factors to encourage suitable migration and integration of cells to the host nervous system? To properly overcome this hurdle, multidisciplinary teams that contain neurosurgeons and biomedical engineers will be essential.

After the proper delivery of cells, a fourth hurdle will be preventing or minimizing oncogenic transformation of transplanted cells. There is a very real concern that undifferentiated patient-derived stem cells when given back to a patient will behave like a tumor. This most likely would behave like a teratoma but even a malignant cancer is

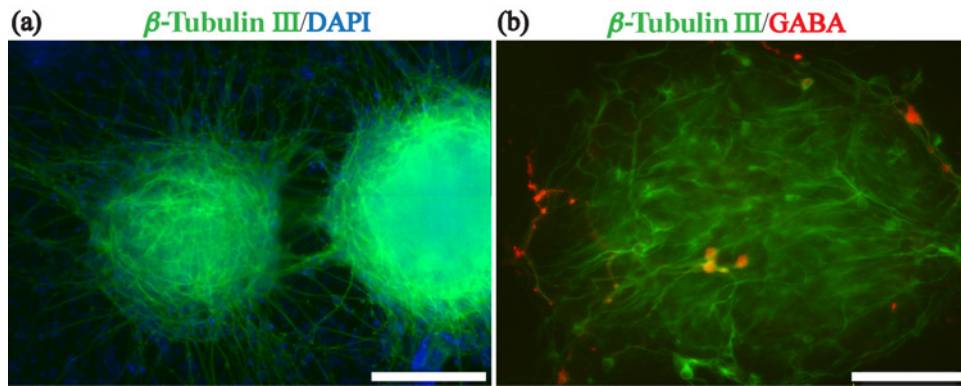


Figure 1 (a) Generation of human neurons from iPSC. A modified Dual-SMAD neuralization protocol^{67,68} was used to generate clusters of cells strongly positive for β -tubulin III (green), a neuronal-specific protein encoded by *TUBB3* gene. DAPI fluorescence (blue) indicates nuclei. Size bar equals 100 μ m. (b) Generation of GABAergic neurons. A differentiation protocol augmenting Sonic Hedgehog signaling was used that biases cells to ventral brain fates including specification of GABA expressing cells (red) that also express the neuronal marker β -tubulin III (green).⁶⁸ Size bar equals 200 μ m. GABA, gamma-amino butyric acid; iPSC, induced pluripotent stem cell; DAPI, 4',6-diamidino-2-phenylindole

possible. The use of more differentiated cells such as NPC or fully differentiated neurons may mitigate this concern if the transplanted cells have a limited capacity for further proliferation. Additional approaches to avoid tumor complications include genetic engineering of patient-derived stem cells to add 'suicide' genes such as thymidine kinase. This would allow transplanted cells to be selectively killed by application of a medication such as ganciclovir. This strategy however is problematic for several reasons including the potential loss of thymidine kinase in transformed cancerous cells rendering this rescue strategy moot. There may also then be inadvertent killing of transplanted cells by accidental exposure to commonly used medications such as ganciclovir. This approach would also contradict the first guideline to avoid the addition of any exogenous DNA as this may lead to immune rejection of transplanted cells (see next hurdle). It should be stressed that there is minimal data to support any of these concerns other than theoretical issues that should be carefully considered by regulatory agencies as well as by scientists, physicians and patients.

A fifth hurdle is to avoid immunorejection of transplanted cells. This last point is generally touted as one of the key advantages of using patient-derived stem cells. This is due to a widely accepted belief that such autologous cells as 'self' will not be rejected by the immune system. While this is may indeed be probable, it has not been rigorously tested and there is some evidence from rodent models that re-programmed cells may still provoke an immune response upon transplantation even leading to rejection.⁴⁷ This may reflect the method of iPSC re-programming, however, as stem cells generated by retroviral transduction most strongly triggered the host immune response.

The future use of patient-derived stem cells needs to be tempered by the many difficult obstacles as detailed above. In addition, severe neurological complications have already been reported after transplantation of human cells as therapies. A boy with ataxia telangiectasia, a genetic disorder caused by mutation of the *ATM* gene,⁴⁸ underwent repeated intracerebellar and intrathecal injections of fetal-derived NPC in an attempt to treat his disorder.⁴⁹

Unfortunately, this child then developed both brain and spinal cord tumors. A partial resection of his spinal cord tumor revealed female cells confirming its origin from a donor.⁴⁹ As another example, a 17-year-old young lady with an aggressive form of multiple sclerosis underwent transplantation with an allograft of MSC isolated from umbilical cord blood as well as autologous MSC derived from her own adipose tissue. She then rapidly became very ill with altered mental status, severe weakness and demyelination consistent with an acquired encephalopathy.⁵⁰ It is unknown whether this adverse clinical course was due to the transplanted allograft or the autologous cells and/or an exacerbation of her underlying multiple sclerosis though she never recovered back to her baseline. Given all of these potential and serious shortcomings, patient-derived stem cells may require many years of testing and monitoring before they can be considered safe and effective therapies for neurological disorders. Their use should also be guided by established ethical principles with very careful consideration of what patients are suitable and what neurological disorders are appropriate for treatment with patient-derived stem cells.

Application to epilepsy

Epilepsy is a worldwide health problem affecting at least 50 million people (WHO Fact Sheet October 2012) and an excellent example of a disorder that may be amenable to modeling and treatment with patient-derived stem cells. Many etiologies for seizure disorders exist, from relatively benign age-related conditions to malignant forms that uniformly are intractable to current treatments and are devastating to patients.^{51–58}

It is estimated that current medical therapies are ineffective for at least one-third of patients with epilepsy. Surgical therapies are possible in some patients but adjacent or intermixed cortex that subserves vital motor or language functions can limit the resection of brain lesions that cause seizures. The application of patient-derived stem cell therapy to epilepsy then appears to be a rational and

practical goal. One can envision ever wider surgical resections to remove epileptic brain regions or brain tumors. In this scenario, even if critical regions of the cerebral cortex have to be removed, we may eventually have therapies to secondarily repair part of the brain. Again, multidisciplinary teams comprised of neurologists, neurosurgeons and biomedical engineers one day may collaborate on cortical resection/regeneration protocols.

Many currently used antiepilepsy medications function by increasing brain levels of gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter.^{59,60} An additional possibility to treat epilepsy would be to transplant GABA expressing neurons that were differentiated *in vitro* from patient-derived stem cells. These cortical GABAergic interneurons normally are responsible for controlling brain homeostasis and the balance between excitation and inhibition of the neural circuitry.^{61,62} Decreased numbers of GABAergic cortical interneurons or decreased GABA neurotransmission can lead to hyperexcitability and seizures.^{63–65} With precedence from animal experiments, it is possible to generate GABA expressing neurons and inject them to the patient's brain.⁶⁶ This approach is somewhat predicated on the inherent ability of cortical GABAergic interneurons to migrate, even in the adult brain. Exogenous application of various growth factors and other compounds may be required to help interneurons to migrate and integrate in targeted cortical regions lacking appropriate inhibition.

As proof of principle for this approach, our laboratory used a 'Dual-SMAD' inhibition protocol to generate relatively pure populations of NPC from human iPSC.^{67,68} These NPC express the radial glia marker PAX6 and upon further neuronal differentiation yielded relatively large numbers of neurons positive for the neuronal specific protein β -tubulin III (Figure 1a). We then added Sonic Hedgehog to our differentiation media to bias production to GABAergic and dopaminergic neurons. These neuronal subtypes are normally specified in the ventral part of the embryonic brain due to high concentrations of Sonic Hedgehog signaling.⁶⁹ While relatively few GABAergic neurons were produced using this protocol (Figure 1b), this example shows how patient-derived stem cells can be used to produce highly specific neuronal subtypes. GABAergic neurons could potentially then be isolated and concentrated and ultimately used for transplantation therapies to treat epilepsy.

Conclusions

Patient-derived stem cells have great potential for both the modeling of neurological diseases as well as *bona fide* treatments. While many hurdles must be overcome before new therapies based on these cells are realized, recent advances give many patients and their families much needed hope. However, hope often coexists with desperation given the often intractable and progressive nature of many neurological diseases. Patient-derived stem cell therapies will then require significant regulatory oversight and much further research at the basic, translational and clinical

levels. This will help ensure both patient safety as well as proven effectiveness to treat neurological disorders.

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