

# New frontiers in human cell biology and medicine: Can pluripotent stem cells deliver?

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Human pluripotent stem cells provide enormous opportunities to treat disease using cell therapy. But human stem cells can also drive biomedical and cell biological discoveries in a human model system, which can be directly linked to understanding disease or developing new therapies. Finally, rigorous scientific studies of these cells can and should inform the many science and medical policy issues that confront the translation of these technologies to medicine. In this paper, I discuss these issues using amyotrophic lateral sclerosis as an example.

Much of modern cell biological discovery has been driven by the study of a diverse variety of primary and transformed cultured cells. However, the advent of human pluripotent cells is providing new avenues of discovery. These cells are genetically manipulable, euploid, expandable to large numbers, and can differentiate to most if not all human cell types. In addition, these cells can be used to analyze the large number of mutations and diverse genetic variation present in large human populations. In fact, not only are human pluripotent stem cells useful for typical cell culture experiments, but they are amenable to many of the types of genetic and molecular genetic approaches that historically have only been feasible in genetic and developmental systems, such as *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Caenorhabditis elegans*, or mouse.

There are two types of human pluripotent stem cells in use. Human embryonic stem cells (hESC) are derived from human embryos that would otherwise be discarded and that are generally donated with substantial informed consent and ethical requirements (Shamblott et al., 1998; Thomson et al., 1998). Human induced pluripotent stem cells (hiPSC) are generated by several different reprogramming technologies, generally from fibroblasts obtained from small skin biopsies or other human somatic cell types, such as blood (Takahashi et al., 2007). Recent work suggests that hESC and hiPSC, although not identical

in their properties, share very important features. First, hESC and hiPSC are both pluripotent so that any cell type of interest can in principle be generated. In fact, differentiation methods for many types of specialized human cells are being developed rapidly, fueled in large part by the need to generate stable differentiated derivatives for cell therapy. Second, hiPSC and hESC can be handled in the laboratory under conditions that are relatively straightforward for skilled cell culture scientists. Third, both hiPSC and hESC, when handled properly, are genetically relatively stable with a diploid karyotype so that gene dose and gene expression at all loci is effectively “normal” or at least representative of expression levels in cells in the intact human. These properties make these cells or their differentiated derivatives suitable for genetic screens using RNA interference, small molecules, insertional mutagenesis, or other analogous tools. Important differences between hESC and hiPSC include an apparent elevation in mutation load in hiPSC (Gore et al., 2011) and differences in epigenetic state. Either of these features may substantially influence the behavior of each cell type and its differentiated derivatives. Thus, hESC and hiPSC may play different roles in the discovery of new cellular and disease mechanisms and in the development of cellular therapies. Recent examples of novel discoveries made using hESC and hiPSC include substantial new insights into the control of the pluripotent state itself and identification of molecular pathways controlling cellular differentiation.

## Disease in a dish approaches with hESC and hiPSC

Although hESC and hiPSC are just beginning to be used to probe and elucidate new cellular processes, there is already substantial progress using pluripotent stem cell approaches to unravel disease mechanisms using so-called disease in a dish paradigms (Unterhaehrer and Daley, 2011). Disease in a dish methods use gene manipulation and/or reprogramming technologies to generate hESC or hiPSC lines with genomes carrying known mutations causing human disease, lesions such as shRNA expression mimicking human disease mutations (Marchetto et al., 2010; Ordóñez et al., 2012), or genomes carrying combinations

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Abbreviations used in this paper: ALS, amyotrophic lateral sclerosis; hESC, human embryonic stem cells; hiPSC, human induced pluripotent stem cells.

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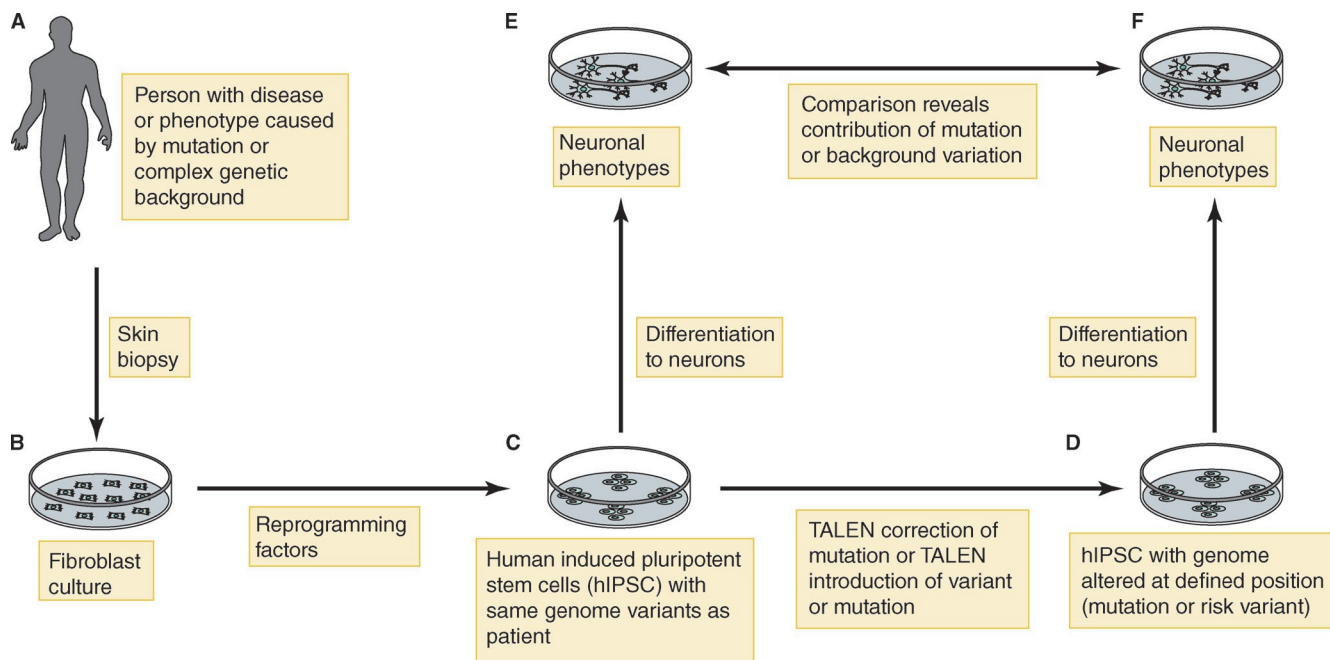


Figure 1. **Disease in a dish.** Stem cells can be used to analyze how human genetic variation or mutation contributes to defined cellular phenotypes. Using neuronal phenotype as an example, Tal effector nucleases (TALENs) can be used to make defined changes in hiPSC of a common genetic background to analyze the contribution of a defined mutation or more complex variation to neuronal phenotypes.

of known or unknown variants that contribute to disease (Fig. 1). With the advent of powerful molecular tools, such as Tal effector nucleases, individual genes can be manipulated by introducing point mutations with great precision (Hockemeyer et al., 2011). Thus, disease-causing mutations or other genetic lesions can be studied for their impacts on cellular processes under “normal” conditions of gene expression and in different genetic backgrounds. Similarly, suppressor and enhancer studies are feasible and will help unravel poorly understood cellular mechanisms. Finally, after differentiation to specialized cell types, cellular mechanisms and interactions as well as disease and potential therapies can be evaluated in bona fide human cells. These approaches are in their infancy but have substantial potential given the limitations of mouse models of disease to accurately recapitulate human disease and the many obvious differences between the details of mouse physiology and human physiology. They also bring unique advantages for diseases in which the key cell types, e.g., human central nervous system neurons, are difficult if not impossible to obtain in good condition or early in disease.

Examples of recent disease in dish studies include a variety of neurodegenerative diseases, heart diseases, and diseases of other organ systems (Unternaehrer and Daley, 2011). Although this work is in its early stages, there is a great deal of potential for meaningful mechanistic cell biological research in this collection of intriguing areas. In addition, these disease in dish models provide unique human materials for direct testing of drug safety and efficacy.

#### **Amyotrophic lateral sclerosis (ALS): A disease in a dish paradigm**

ALS, also known as Lou Gehrig’s disease, provides an intriguing example that illustrates the path from mechanism-based research

to understanding and potential therapies using disease in a dish approaches. ALS is a disease in which death of motor neurons causes paralysis of voluntary muscles. As the disease progresses, paralysis ultimately extends to the muscles involved in breathing, swallowing, and all other voluntary movements. Once diagnosed, ALS generally causes death within 3–5 yr or less. There is only one approved drug for ALS, riluzole, but individual patients do not perceive much benefit because riluzole generates statistical prolongation of life for only a few months based on large-scale clinical trials. The key problem of course is to learn what causes death of motor neurons in ALS and whether this information might help develop a therapy that protects motor neurons from dysfunction and death.

Although most ALS is “sporadic,” some forms are hereditary, including a form caused by mutations in the gene encoding dismutase SOD1 (superoxide 1). The generation of transgenic mouse and rat models that carry human mutant SOD1 genes has led to substantial progress in the understanding of cellular mechanisms that contribute to disease. In particular, a series of genetic studies in transgenic and chimeric mice led to the realization that the death of motor neurons in ALS might not be cell autonomous (Clement et al., 2003; Boillée et al., 2006; Yamanaka et al., 2008a,b). Disease in a dish studies using astrocytes carrying SOD1 mutant genes mixed with in vitro differentiated motor neurons made from pluripotent stem cells confirmed these findings and lead directly to searches for secreted toxic factors and drug testing (Di Giorgio et al., 2007, 2008; Marchetto et al., 2008). The general conclusion from these studies is that motor neuron death in ALS is strongly influenced by other cells in the spinal cord that make important contributions to, or protect from, motor neuron death. Whether astrocytes, microglia, or other cell types carry

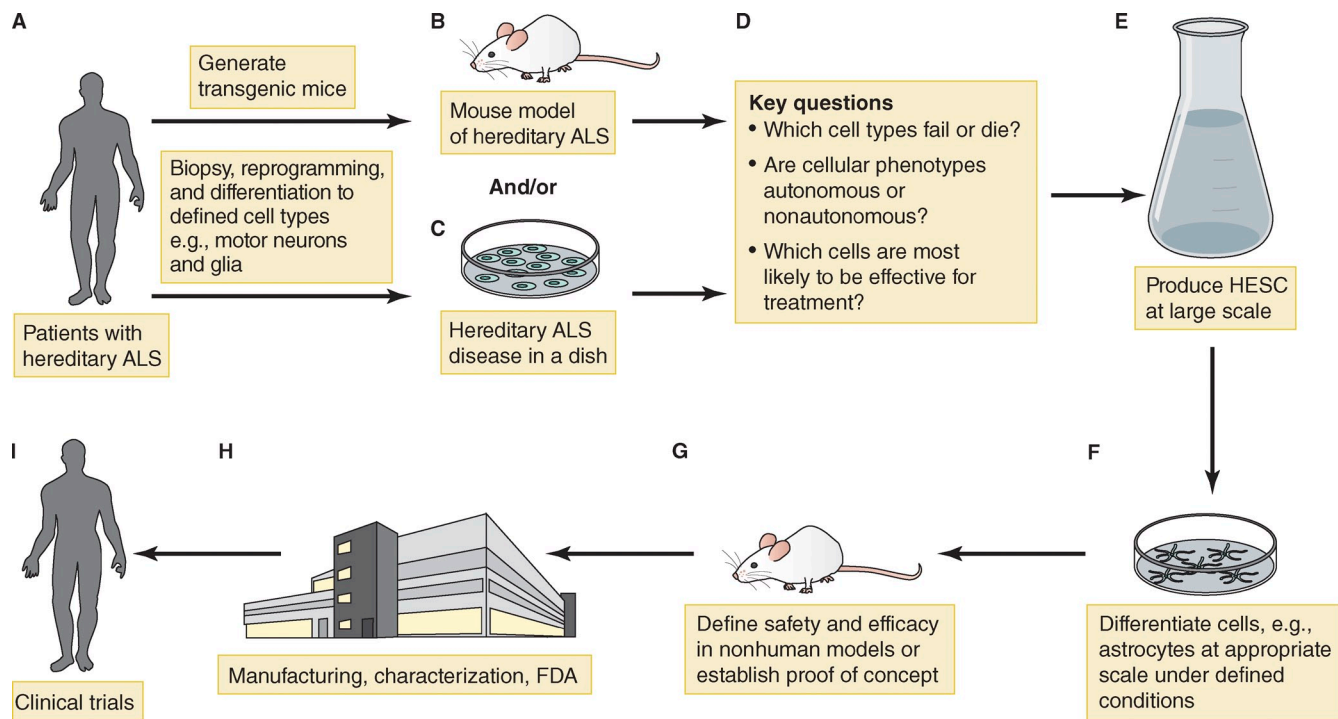


Figure 2. **Cell replacement therapy using stem cells.** A possible path from patients with hereditary ALS to cell biological research using transgenic mouse or pluripotent stem cell models to cell therapies. In this example, assessing the contribution of different cell types to ALS through rigorous research can lead to a rational approach to cell therapy.

mutant SOD1 genes determines whether they exhibit stimulatory or protective effects on motor neuron death. Although such conclusions might be limited to SOD1-mediated ALS, there is also recent evidence that astrocytes might contribute to sporadic ALS as well (Haidet-Phillips et al., 2011).

#### Development of cell replacement or augmentation therapies

Successfully treating debilitating and currently incurable diseases with cell replacement or augmentation therapies requires basic cell biological research to fuel the generation and testing of new therapies (Fig. 2). For example, expansion of hematopoietic stem cell therapeutic approaches from leukemias to other diseases is based on a sound understanding of the basic cell and developmental biology of these cells. Several different stem cell therapies are in the midst of development and testing for several disorders, including spinal cord injury, graft versus host disease, skin diseases, blindness, diabetes, and AIDS (e.g., California Institute for Regenerative Medicine, 2009; Pollack, 2012; Schwartz et al., 2012). Using ALS as an example, some stem cell-based therapy efforts are aimed at trying to replace motor neurons that are damaged or die in ALS. But inducing motor neurons or their stem cell precursors to engraft into spinal cords or upper motor cortex and then appropriately extend axons and wire to peripheral muscles may be a challenge that will take many years to solve. Interestingly, the evidence that ALS is non-cell autonomous with major contributions from astrocytes and other glia has led to two different categories of cell therapy approach. One approach, which I regard as little more than guesswork, has

tried to treat ALS by introducing poorly defined mesenchymal stem cells or cord blood stem cells directly into the spinal cord of ALS model animals. In fact, even in the absence of strong and reliable evidence, a clinical trial of cord blood stem cells transplanted into the spinal cord of human ALS patients was launched by a private company ([http://www.tacellulartherapy.com/fda\\_clinical\\_trials.html](http://www.tacellulartherapy.com/fda_clinical_trials.html)) and then halted by the FDA. A more rational approach given the state of scientific understanding, the state of experiments in animal models, and the in vitro data is to introduce progenitor cells that can differentiate to astrocytes or progenitors that secrete growth factors (Klein et al., 2005; Suzuki et al., 2007; Lepore et al., 2008; Suzuki and Svendsen, 2008; Hefferan et al., 2012). One of these approaches has recently reached clinical trials using fetal-derived spinal cord stem cells in which one hopes that enough will be learned to support more trials using different stem cell-generated preparations and perhaps different surgical methods or spinal cord sites.

#### Driving evidence-based scientific and medical policy with stem cell-driven discovery

The social and medical issues that arise in the development of cell therapies are and will be heavily influenced by the scientific discoveries about and using human stem cells. These social and medical challenges are well illustrated by a discussion of ALS owing to its rapidly progressive and devastating nature.

First is whether one type of therapy can treat all types of ALS patients. Solving this issue will require a better understanding of what causes ALS, what cellular mechanisms contribute to

motor neuron death, and which cells contribute in different forms of ALS. One key and possibly false assumption that drives current efforts is that all forms of ALS will exhibit the type of cellular nonautonomy found in animal models of SOD1-mediated ALS. Thus, models of sporadic ALS and hereditary forms of ALS such as those mediated by FUS/TLS or TDP-43 mutations must be tested. These experiments will also allow tests of the magnitude of the relative contributions of different cell types to motor neuron death or rescue in different forms of ALS. Additionally, if the actual cellular pathways that are defective in astrocytes and motor neurons can be better defined, cellular augmentation strategies and drug discovery could take advantage of that information.

Second is the so-called snake oil problem (<http://www.closerlookatstemcells.org>; CBSNews, 2010). Numerous misleading and probably fraudulent advertisements can be found about clinics offering stem cell cures for ALS. These wild claims ignore large amounts of scientific data about the nature of ALS and rational approaches to therapy and prey upon those who don't have ready access to or cannot evaluate legitimate scientific and medical information. Our legitimate scientific and medical community needs to stand against these frauds and provide accurate information derived from rigorous research to patients so that they are not taken advantage of by these clinics. In addition, we must work to ensure that legitimate efforts are not damaged by the blowback from those who effectively steal from desperately ill patients and their families or the likely harm to these patients that is coming from clinics that dispense untested and sometimes dangerous therapies.

Third is the cell tracking problem. Currently, it is difficult to know how cells transplanted into the spinal cord of an ALS patient behave until after a patient has died. In addition, using antibodies to examine postmortem material from a transplant patient is problematic because the transplants are of human cells into a human patient. We desperately need to develop safe and sensitive methods for cell marking and imaging that will allow us to track cell behavior in patients in real time after transplant. Real-time measures would allow therapy to be modified or even repeated based on the analysis of cell behavior. These methods will rely heavily on cell biological research to identify cellular pathways and markers that could be visualized in real time by magnetic resonance imaging, positron emission tomography, or other imaging modalities.

Fourth is how to manage individual patient response versus the average response of patients in a clinical trial. Although most often thought about with respect to drug therapies, different forms of ALS might vary in their response to cell therapies. An interesting possibility is that hiPSC lines from individual patients could be used not only for drug testing but also for evaluating the genetically driven contribution of different cell types to each patient's version of ALS. A corollary is that for ALS patients included in a clinical trial, the notion that cells would be introduced only once and that the patient would then be "passively" followed with no change in treatment paradigm until death might be unacceptable. In conventional medicine, one might try treatment again or modify treatment course, depending on how an individual patient responds. On the

other hand, prospective design of a statistically rigorous clinical trial requires development of a treatment plan and identification of rigorous outcome measures that should not be modified if the data are to be interpretable. Development of new statistical methods, outcome measures, hiPSC evaluation of phenotypes, and perhaps, cellular marking methods might allow trials to tolerate modification as part of an ALS patient's clinical care. Perhaps rigorous data from hiPSC-based research could be used to make a case to the FDA that ALS clinical trials need to be more responsive to patient needs and variable outcomes with a disease that is as complicated and clinically inconsistent as ALS.

Fifth, and finally, is the risk benefit analysis that can hinder or accelerate the development of therapies for rapidly fatal diseases such as ALS. Current paradigms of therapy development are risk averse and require enormous amounts of information on safety and possible efficacy before trials can be approved, financed, and launched. Yet, some patient populations, such as those with ALS, when facing a near certain death sentence, are very risk tolerant and might be willing to participate in trials with much lower certainty. Our community must work with the FDA to tackle this problem and to perhaps dramatically accelerate the introduction of good, but perhaps radical, ideas that might work but in which safety or efficacy testing in animals could take many years, or simply be unreliable, so that current patients would have no hope of benefiting. I often ask myself, as I work with my colleagues to develop a cell-based therapy for ALS that has been partially tested in animals but is not yet complete and therefore not ready for humans, what I would do if I, my wife, or one of my children developed ALS. Would I be willing to have appropriate types of stem cells or their derivatives transplanted into them even if I were not absolutely certain and had not yet proven absolute safety or efficacy? Interestingly, I find that in thinking about this issue, I fall back on my scientific understanding of ALS and the rigorous types of data on ALS mechanisms in the mainstream scientific literature. The result is that my own risk tolerance rises substantially when I have the ability to consider published and unpublished data and how it might be applied. I think that all ALS patients should have this information and that the FDA should be responsive to these patients when they want to take well-informed risks with experimental therapies that may not yet meet current FDA standards. Clearly, the devil will be in the details for implementing such an approach and ensuring patient protection as well as opportunity, but we owe this consideration to current ALS patients and those with comparably severe diseases. Again, however, this is a debate in which rigorous scientific research can drive the agenda and resulting policies.

### Concluding remarks

Virchow developed the concept that disease arises in the individual cells of a tissue (Schultz, 2008). This important principle is the foundation for using human stem cells to understand basic cellular mechanisms and to extend that understanding to the development of therapies. Treating disease by targeting the misbehaving cells is clearly a wonderful opportunity for therapy



development and research. Thus, probing the secrets of human cells by taking advantage of human pluripotent stem cells may signal the dawn of a new era in cell biology research.

Finally, consider the many remarkable discoveries and novel mechanisms found when the basic tools of cell and molecular biology were applied to unusual members of the model organism toolbox, including snakes, ciliates, planaria, jerboa, and other organisms that have developed unusual biological strategies during evolution. Could humans be added to this list, and could the study of basic human cell biology yield comparable discoveries? Because humans are a large, long-lived organism with a complex brain, a rich evolutionary history, and substantial genetic variation across large and accessible populations, the answer is certain to be yes.

I apologize to the many authors whose work I could not cite because of space considerations.

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