Translating the Lessons From Gene Therapy to the Development of Regenerative Medicine

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One of the more exciting advances in the past decade has been the surprising discovery by Takahashi and Yamanaka that primary somatic cells can be converted into pluripotent cells (induced pluripotent stem (iPS) cells) by the forced but transient expression of a small number of defined transcription factors, a discovery that may well prove worthy of a Nobel Prize.[1] While there is significant excitement about the study and the therapeutic potential of human embryonic stem (ES) cells, the ethical issues surrounding the destruction of embryos that is necessary to generate such cells has slowed scientific investigation into their use. Because iPS cells are generated without the need to destroy an embryo, their discovery has further energized the field of regenerative medicine and stem cell biology. Indeed, there are clear similarities between the excitement generated by iPS cells and regenerative medicine today and that generated by the advent of gene therapy several decades ago. One hopes that the lessons learned from the growing pains experienced by the field of gene therapy will be applied by the leaders of this new field so as to hasten and facilitate the clinical translation of safe iPS cell technology. In this Commentary, I focus on several areas where these lessons can be applied to the iPS cell field.

Although the degree to which somatic cells can be reprogrammed to a pluripotent state is variable, it nevertheless appears that the regenerative and differentiation potential of iPS and ES cells is quite similar.[2,3] ES and iPS cells can both be propagated indefinitely and induced to differentiate into a wide range of cell types *in vitro*. Importantly, since they can be generated from a range of easily accessible cell types, iPS cells can be derived from essentially any individual, rendering them a powerful reagent for scientists across a broad range of disciplines. Moreover, since iPS cells are a relatively new scientific commodity, there are likely to be many uses for them that have yet to be contemplated. Nonetheless, there are at least four current areas of research for which iPS cells have clear importance, which I outline below.

The ability to reprogram differentiated somatic cells into pluripotent cells using defined transcription factors allows scientists to study the mechanisms by which the stem cell state is established, maintained, and lost. Similarly, the ability to differentiate iPS cells *in vitro* into a wide variety of more specified cells allows workers to study the specification and differentiation of human somatic tissues more easily than previously possible. It is not possible, for example, to visualize the differentiation of human cells into mature neurons *in vivo*, and the ability to differentiate iPS cells into neurons *in vitro* provides a powerful new approach to studying such processes.

The ability to derive iPS cells from any individual also means that they can be—and are being—derived from patients with any disease. These human disease–specific iPS cells provide a unique and previously unavailable resource for studying the pathophysiology of various important human diseases. Given the subtle heterogeneity in different iPS cell lines, however, care will need to be taken to ensure that differences found between patient-derived cell lines and normal cell lines are attributable to the underlying pathogenesis of the disease rather than to idiosyncratic differences between individual iPS cell lines. A simple guideline might be that any differences observed be confirmed in iPS cell lines derived from at least two unrelated patients.

The ability to differentiate iPS cells into a wide range of primary human cell types, many of which are unavailable for routine use, also provides a tremendous resource for drug development. One can imagine the development of a collection of iPS cell lines representing a variety of genetic and ethnic backgrounds. Such a collection could then be differentiated into a panel of human primary somatic cell types that would, at least partially, encompass the range of genetic variation in humans. Using high-throughput screening, this panel could then be used as a platform to screen small molecules for desired phenotypic effects in primary human cells. Moreover, this panel could serve as a platform for preclinical toxicology screens on primary human cells in a way that is also not otherwise currently possible.

Finally, iPS cells have tremendous potential in regenerative medicine. There are two basic visions of the possible therapeutic use of iPS cells. The first is their use as a source of unmodified cells to replenish a degenerating tissue or organ. Although the direct therapeutic application of iPS cells is not possible because they develop into teratomas, the generation of transplantable somatic stem cells from iPS cells (neural stem cells, for example) may offer a strategy for organ regeneration. The second vision entails genetic modification of patient-derived iPS cells and then transplantation of these genetically modified cells back into the patient for therapeutic purposes. Hanna *et al.*[4] published a seminal proof-of-principle study in mice that demonstrated how one might combine homologous recombination with iPS cell technology to cure a human genetic blood disease.

The very formidable challenge in both approaches is to develop safe and effective transplantation protocols for iPS-derived cells. This challenge may take decades to overcome, as the only stem cell

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we currently know how to successfully transplant in humans is the hematopoietic stem cell. Therefore, figuring out how to generate sufficient numbers of safe somatic stem cells from iPS cells and transplant them with clinical benefit is likely to be a long-term endeavor. Substantial progress has been made in deriving iPS cells in which the oncogenic reprogramming factors are no longer present. A more subtle issue, however, is whether the reprogramming process itself creates an epigenetic state that predisposes any progeny of these cells to transform into cancer. It is possible that under certain circumstances the direct transplantation of cells will not be successful and, instead, the cells will need to be transplanted after being embedded within a biomaterial. Given that there is no current example of such a treatment, this strategy is also likely to take decades before it becomes a useful therapy. A further challenge for using genetically modified iPS cells for therapy is determining how to create such cells in a safe fashion. Strategies involving homologous recombination or the isolation of genetically modified iPS clones in which the transgene has integrated into a safe harbor represent two possible ways of ensuring that the genetic modification of the iPS cells is accomplished in a fashion that is as safe as possible.[5–7]

An important problem that has plagued the gene therapy field and that will pose similar challenges to the field of regenerative medicine is the development of appropriate preclinical models. As the statistician George Box proclaimed, "All models are wrong but some are useful." Despite the utility of using mice as a model for iPS cell–based therapies, it is important to recognize their limitations. Barrett and Melenhorst recently discussed this issue in an elegant Commentary in this journal,[8] underscoring that mouse and human physiology is different and that laboratory mice are inbred genetically homogeneous populations whereas humans are outbred and genetically heterogeneous. In addition to the physiological and genetic problems with mouse models, there is the simple problem of scale. A normal adult human is more than 3,000 times larger and lives ~35–40 times longer than a mouse. The shorter life span of laboratory rodents, for example, changes the dynamics of cell generation and replacement throughout life.[9] This scale difference has important ramifications for both efficacy and safety as investigators will need to safely generate over 3,000 times more cells to transplant. The gene therapy trials for severe combined immunodeficiency-X1 provide an example of how scale can lead to problems in human trials—leukemia did not develop in patients until two to three years after the infusion of retrovirally modified cells, a time point exceeding the life span of an experimental mouse.[10]

Indeed, the safety of any iPS cell–based therapy is a paramount concern. An important advantage of such an approach is that a single cell can be expanded to generate a large population of cells, suggesting that one could perform a complete sequence analysis of the original clone so as to determine its genetic safety. Unfortunately, however, the *in vitro* expansion of a clone—even if the cancer-predisposing reprogramming factors have been eliminated—can select for both epigenetic and genetic events that predispose the progeny to cancer, particularly when generating sufficiently large numbers of cells to treat a human disease. Several recent publications have described the genetic instability of iPS cells grown in culture.[11,12] Moreover, such genetic instability might also antagonize the subsequent ability to differentiate the cells into therapeutic, transplantable cells—decreasing the efficacy of the treatment as well. It is therefore critical to develop better methods of expansion to prevent even a very small number of cells from acquiring genetic and epigenetic changes that might lead to cancer following transplantation into patients.[13]

In addition to the use of homologous recombination as a way of safely marking and modifying iPS cells as discussed above, several other tools developed by the gene transfer community are likely to be of use in regenerative medicine. One could use suicide genes to label iPS-derived cells prior to transplantation so as to eliminate the cells if they were found to cause harm in a patient. In addition, one could genetically modify iPS cells with transgenes—either protein-coding genes or micro-RNAs—that could direct or facilitate differentiation of an iPS cell into a specific transplantable cell type and/or inhibit its differentiation into an undesired cell type. Introduced transgenes might also contain tissue-specific regulatory elements to allow positive selection when iPS cells are differentiated into the desired cell type and negative selection if they were to differentiate into an undesired cell type. Of course, the introduction of these transgenes into iPS cells would need to be done in a way that did not compromise the safety of their subsequent use.

It would be unfortunate if the recommendations of the Orkin and Motulsky report (http://www.nih.gov/news/panelrep.html) regarding gene therapy were not heeded in the field of regenerative medicine and if clinical trials were performed without the benefit of learning something from any failures. Indeed, the history of the development of solid-organ transplantation, hematopoietic stem cell transplantation, and the continued development of gene therapies suggests that the early clinical trials for iPS cell–based therapy may very well be clinical failures. An important aspect of early iPS cell–based trials is that, while the primary end points will focus on patient safety, secondary end points should be built into the studies so as to facilitate understanding of the mechanisms responsible for any failures. Because these studies are likely to be performed using iPS-derived autologous cells, it will be impossible to distinguish the transplanted cells from untransplanted cells unless the transplanted cells are marked in some way. The gene therapy field has established that it is both feasible and ethically permissible to use gene marking in early clinical trials. It seems prudent, therefore, that safe ways of marking iPS-derived transplanted cells and sensitive methods to identify these cells be developed and used in order to track the fate of the transplanted cells.

Given the sometimes ethically contentious nature of pluripotent stem cell research, a demonstration of strict self-regulation with a forum for public input and comment will be an important aspect in maintaining the long-term public trust and support of the field as it goes through its inevitable growing pains. The Recombinant Advisory Committee (RAC) was originally formed as a centralized forum to evaluate the safety of experiments involving recombinant DNA. The RAC has

since evolved into a centralized forum for the review of gene therapy trials. Although the RAC is not perfect, a similar review committee should be formed within the Department of Health and Human Services with oversight for iPS cell–based therapies. Such a forum for clinical trial review will support the development of expertise in reviewing these trials that would be almost impossible to match with local institutional review boards. In addition, approval from a respected centralized review committee would provide a degree of imprimatur for the studies. Studies approved by this committee could be listed on publically accessible websites, such as those of the American Society for Gene and Cell Therapy and the International Society for Stem Cell Research, so that the public could easily identify the studies that have passed careful scientific scrutiny. Finally, a review at the federal level with open publication of the proceedings and deliberation would provide transparency to the public and research communities.

Finally, the public and private excitement surrounding the development of iPS cells for regenerative medicine can lead to a potentially troublesome bias. Given the systemic incentives for all stakeholders in the field—including researchers, journals, funding organizations, biotechnology companies, and patient groups—there is a natural urge to focus on the positive, headline-grabbing advances. Some of the difficulties of gene therapy have arisen from unrealistic predictions and expectations for the evolution of laboratory-based studies into standard therapy for patients. Similarly, there is an understandable desire to avoid doing experiments that might undermine the field. Yet, in many ways, experiments that elucidate the risks and barriers to translation are exactly those that should be undertaken first because they will identify the problems that need to be solved before the technology can be applied to improve treatment for patients. This early and thorough identification of problems is important to stimulate creative solutions. These solutions will potentially arise from junior investigators and investigators outside the formal stem cell community, as these groups are less encumbered by biases and assumptions within the field and thus could be more flexible in their thinking about potential solutions.

In summary, the developing field of regenerative medicine is exciting on many levels, not the least of which is the possibility that it might lead to improved therapies for patients. The recent history of the development of gene therapy highlights several important lessons that can be used to hasten and facilitate the development of therapeutic regenerative medicine. An active, free, and open discussion, including support for careful research into the risks of iPS cell technology in clinical applications, is one way that the lessons can be effectively communicated, processed, and applied.

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