Career Moves

Induced Pluripotent Cells From Human Aortic Smooth Muscle Cells Can Efficiently Redifferentiate Into Parental Phenotype

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The available evidence demonstrating improvement in myocardial function following transplantation of autologous bone marrow–derived stem/progenitor cells, in both preclinical and available clinical trials, remains a potent force driving discovery and clinical development simultaneously and has provided new hope for patients with debilitating heart diseases. However, the availability of adult stem cells that can regenerate multiple cell types, including heart muscle, remains a significant challenge, and availability of a more plastic, pluripotent autologous stem cell source that can regenerate microvasculature and large vessels, as well as lost cardiomyocytes, is continuously being pursued. The inherent plasticity of embryonic stem cells (ESCs) is, therefore, argued to be an advantage for their potential application in regenerative medicine. Although ESCs have been used in animal studies of cardiac repair, ethical, technical, and regulatory issues, as well as unavailability of autologous human ESC for cell therapy applications, limit the potential therapeutic utility of ESC in humans. In this regard, the recent advent of somatic cell reprogramming to generate induced pluripotent stem cells (iPS) has generated significant enthusiasm and has opened avenues for development of patient specific autologous, multipotent cell source for regenerative medicine.

The remarkable discovery by Yamanaka and colleagues that adult cells can be reprogrammed to become ESC-like iPS cell by retroviral transduction of defined transcription factors, Oct4, sox2, klf4, and c-myc has revolutionized stem cell research and led to the generation of mouse and human iPS cells from different laboratories. Within 3 years of this original discovery, the field of iPS cells has moved forward with unprecedented speed. Many of the shortcomings of the original approach, such as use of retrovirus that may integrate into the reprogrammed cells genome, use of an oncogene as one of the reprogramming factors, etc, have been overcome: reprogramming can now be achieved with plasmid transfection of defined factors, without oncogene insertion and with as little as 2 factors. The generation of iPS cells by adenovirus or recombinant protein, as well as the use of cell and transgene-free ESC protein extracts, a methodology developed in our laboratory, has further alleviated certain original concerns regarding the potential use of iPS cells in regenerative medicine.

It is now well established that iPS cells can differentiate into all 3 germ layer–derived cells. These cells are syngeneic, indicating that they can become an ideal cell source for regenerative medicine. However, detailed differentiation properties and the directional differentiation system of iPS cells have not been demonstrated. Taura et al determined the features of the directed differentiation of human iPS cells into vascular endothelial cells and mural cells and compared that process with human ESCs. The authors succeeded in inducing and isolating human vascular cells from iPS cells and showed that the properties of human iPS cell differentiation into vascular cells are nearly identical to those of human ESCs. Zhang et al successfully characterized the cardiac differentiation potential of human iPS cells compared to human ESCs. Electrophysiology studies indicated that iPS cells have a capacity like ESCs for differentiation into nodal-, atrial-, and ventricular-like phenotypes based on action potential characteristics. Both iPS cell– and ESC-derived cardiomyocytes exhibited responsiveness to β-adrenergic stimulation manifested by an increase in spontaneous rate and a decrease in action potential duration.

Smooth muscle cell (SMC) differentiation and dedifferentiation play a critical role in the pathogenesis of cardiovascular diseases. Xie et al successfully differentiated mouse iPS cells into SMCs in vitro. Also, the iPS cell–derived SMCs acquired SMC functional characteristics including contraction and calcium influx in response to stimuli. However, a comparative analysis of the molecular, epigenetic, and biological properties of cells differentiated from iPS cells and those of the same lineage of somatic cells from which the iPS cells originated is essential to understand the translational potential of these cells.

In this context, the study by Lee et al in this issue of *Circulation Research* is important and attempts to address this concern. Lee et al generated iPS cells from human aortic vascular SMCs (HASMCs) and redifferentiated these iPS cells back into SMCs. The authors characterized and compared the gene expression and cellular functionality of iPS cell–derived SMCs with the parental HASMCs from which the iPS cells were derived. Established iPS cells were shown to possess properties equivalent to human ESCs, in terms of the cell surface markers, global mRNAs, and microRNA expression patterns, epigenetic status of OCT4, REX1, and NANOG promoters, and in vitro/in vivo pluripotency. The cells were differentiated into SMCs to enable a
Consistent with ESCs, the exposure of iPS cells to such multiple factors that is critical for proper development. Systems; it is the time- and context-dependent expression of organ induction during the development of multiple organ factor that acts constantly throughout the entire process of ESCs into cardiomyocytes.16,17 There is no single growth heart development in a context- and time-dependent manner. Although several signaling protein families are involved in various growth factors and corresponding inhibitors to heart evolution of microvascular repair and collateral artery growth is a promising alternative approach for noninvasive treatment of arterial obstructive disease, such as coronary, peripheral, or cerebral artery disease. With the growing knowledge of the mechanisms involved and the factors that influence these processes, an increasing number of clinical trials are being performed to stimulate neovascularization. The expression of growth factors and the cooperation of surrounding and infiltrating cells seem to be essential in orchestrating the complex processes involved. In light of the above developments, attempts to stimulate the growth of collateral arteries using the iPS cells described by Lee et al15 might ultimately lead to new treatment options for patients with vascular occlusive diseases.

However, caution should be used while determining whether differentiated cells derived from iPS cells, such as SMCs, have the same functional properties as their physiological in vivo counterparts. Also, it is important to determine the factors involved in maintaining such physiological functions in vivo. Recent studies describe the contribution of various growth factors and corresponding inhibitors to heart development during embryogenesis. Bone morphogenetic proteins, Wnt protein, and Notch signals play critical roles in heart development in a context- and time-dependent manner. Although several signaling protein families are involved in the development of the heart, limited evidence is available about the exact signals that mediate the differentiation of ESCs into cardiomyocytes.16,17 There is no single growth factor that acts constantly throughout the entire process of organ induction during the development of multiple organ systems; it is the time- and context-dependent expression of multiple factors that is critical for proper development. Consistent with ESCs, the exposure of iPS cells to such growth factors is hypothesized to augment differentiation into cardiomyocytes.18 Use of such appropriate developmental signal information for functional blood vessel development using iPS cells has the potential for providing the foundations for future regenerative medicine.

Several issues will need a more comprehensive resolution before iPS cells can find clinical applications. Many of these issues are, in fact, not unique to iPS cells but have already been noted for human ESCs as well. Tumorigenicity remains a major concern in the use of iPS cells in regenerative medicine. Making a somatic cell pluripotent predisposes that cell to cause a tumor. There are compelling reasons for worrying about iPS cell tumorigenicity based on actual published data. Of greatest concern is that nearly all iPS cells described in published studies have been demonstrated to cause teratomas, proving pluripotency but also tumorigenicity, and that mice genetically derived to contain some tissues from iPS cells exhibit an incidence of malignant tumors. Genetic changes inherent in the iPS cell generation process may pose risk of enhancing tumorigenesis both through the introduced genes themselves and, in theory, via potential changes at specific integration sites. Moving away from methods of induction that rely on genetic changes (eg, using protein factors rather than exogenous retrovirus based nucleic acids transduction) may reduce the tumorigenic potential, but this remains to be confirmed. Another question that needs a more comprehensive analysis is whether all iPS lines derived from the same parent somatic cells are exact epigenetic and molecular signatures or whether they vary from one another to a certain extent and whether these variations influence their tumorigenicity and developmental potential. Additionally, the long-term stability of induced pluripotency and epigenetic status of a given iPS cell line needs to be validated.

Mostly under the radar in the field of stem cell safety are potential undesirable side effects of epigenetic changes in the developed iPS cell lines. Because epigenetic changes are postulated to play a key role in the reprogramming and are at the heart of iPS cell formation, it is critically important to more fully study the global epigenetic changes associated with pluripotency and especially induced pluripotency. An ever-changing or unstable epigenome can pose an unanticipated risk of enhancing tumorigenesis. Characterizing the relationships among the epigene, pluripotency, and tumorigenicity should prove of great benefit for developing safe regenerative medicine. The road from where we are today to a future with iPS and human ESC–based regenerative medicine therapies being safe and more common treatment modalities is not a clear, linear one. Beyond tumorigenicity issues, it still needs to be determined how comparable the in vitro–derived cell types are to their in vivo counterparts and how to isolate them at sufficient purity. Regardless of the type of stem cells used, high-quality transplantation methods using tissue engineering must be developed. The existing and developing evidence from fields as diverse as developmental biology, stem cell biology, and tissue engineering must be integrated to achieve the full potential of iPS cells in cardiovascular regenerative medicine. Moreover, a general and efficient way of characterizing large numbers of human iPS cell– and iPS cell–derived cells also needs to be develop-
oped to provide a routine, high-throughput method for quality control and for their immediate application in drug screens and basic research.

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