Quill & Scope

Volume 4 *Volume IV* Article 22

2011

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Since, the isolation of human embryonic stem (hESCs) cells by Thomson *et al.* in 1998, stem cell research has been recognized as the most promising component of the regenerative medicine.[1] Stem cells are capable of differentiating into any cell type of the body and this pluripotency allows for limitless therapeutic potential. However, the utility of hESCs is restricted because of pertinent ethical issues, limited availability of human blastocysts, and possible allograft rejection. Furthermore, our understanding of cellular differentiation and epigenetic regulation are still in the primitive stages and pose major hurdles in stem cell research. Before stem cell therapy can become a clinical reality, it is imperative that we address critical issues relating to programming efficiency, tumor susceptibility, and graft survival and rejection. Nonetheless, commendable milestones have been already achieved in the study of stem cells by a number of research groups, and regenerative medicine is gradually inching towards a plausible reality of stem cell therapy. The FDA has recently approved the usage of a hESC-based therapy in 2010 where clinical trials have been initiated by Geron Corporation for patients with spinal cord injury. In this article, we appreciate the recent advancements in stem cell research and discuss current challenges faced by various research groups in stem cell research.

The discovery of induced pluripotent stem cells (iPSCs) has paved a new paradigm in stem cell research. The exemplary ingenuity of Shinya Yamanaka in developing a cellular reprogramming technology that facilitates production of iPSCs from somatic cells provided a major breakthrough in stem cell research[2]. Yamanaka hypothesize that transcription factors playing important roles in the maintenance of hESC identity also have pivotal roles in the induction of pluripotency in somatic cells. The research group identified numerous factors that are differentially expressed in hESCs in comparison to adult stem cells. By performing detailed comparative analysis, they defined four key transcription factors, namely Oct3/4, Sox2, c-Myc, and Klf4, that are essential to the reprogramming of somatic cells. This landmark finding lead to the realization of a long awaited scientific dream that adult somatic cells can be reprogrammed into induced stem cells and perhaps be used for therapeutic purposes.

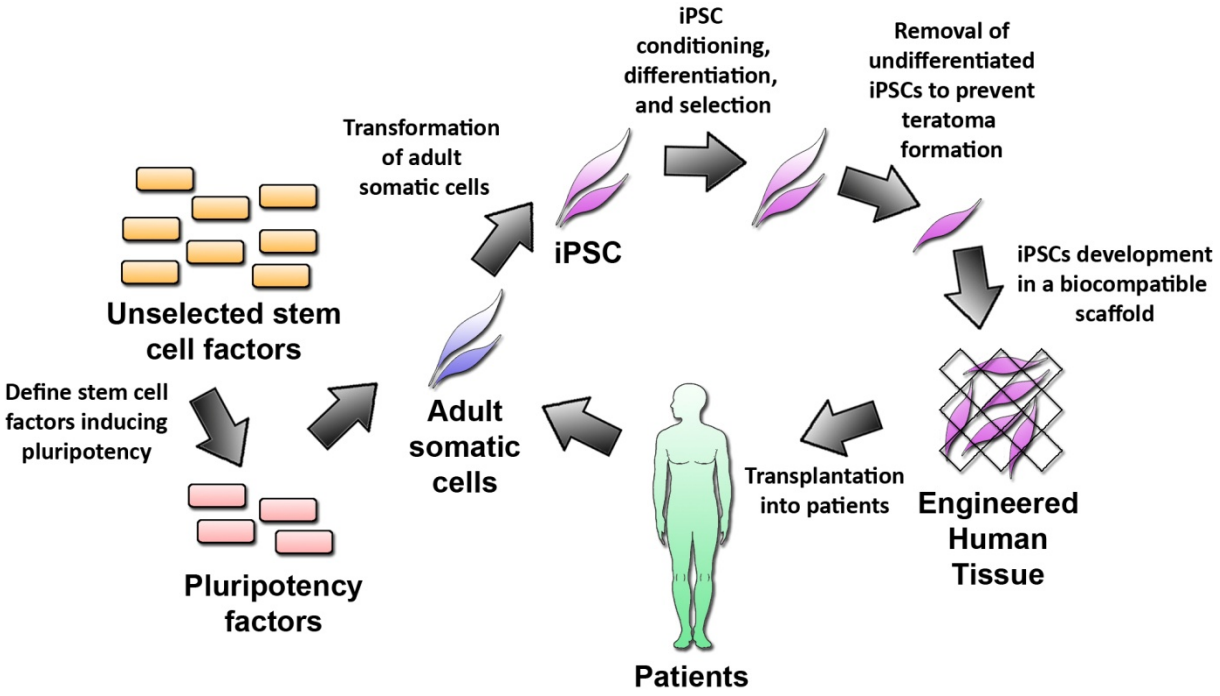
Despite the efforts of Yamanaka in pioneering iPSC technology, several limitations immediately became apparent[3]. The transgenes used for reprogramming were based on retroviral vectors with random insertion into the host genome, allowing for possible interference with the endogenous gene structure that could result in undesirable insertional mutations. Furthermore, the lack of complete control of transgene expression could result in tumorigenesis. Several solutions have been explored to address these issues including the use of cell-penetrating recombinant proteins for reprogramming cells.[4,5]

Initial studies exploring cellular reprogramming with Oct3/4, Sox2, c-Myc, and Klf4, have led way to the discovery of a multitude of additional transcription factors that can reprogram iPSCs and in some cases alter the eventual differentiation of cells.[6,7] Alterations in chromatin structure affecting epigenetic regulation are important, dynamic regulators of global genetic expression in iPSCs but remain an uncharted territory[8] Disruption of tumor suppressor p53 or cell-cycle checkpoint inhibitors (e.g. p14/ ARF, p21) can improve iPSC generation, however are also involved in carcinogenesis.[9-12]

Human iPSCs have been generated from fibroblasts, keratinocytes and CD34+ human peripheral blood cells[13,14]. Recent studies have indicated that cell type and culture environment can affect iPSC generation. While certain cell types may prove to be easier to extract cells from, such as fibroblasts from a skin biopsy, other cell types may be more effective for tissue development. Development of iPSCs from keratinocytes show lower rates of transgene retroviral insertion than fibroblasts thus allowing for more regulated control of cell genetics.[13] The low efficacy of iPSC generation *in vitro* also

remains an important challenge for the generation of significant tissue yields.

Evaluation of generated iPSCs for therapeutic efficacy and tumor formation is necessary prior to implantation in patients. In this regard, animal models will be important to assess germline transmission of iPSCs and cell incorporation into patient tissue. Furthermore, animal models prior to clinical development will be important to screen for the formation of teratoma tumor masses from undifferentiated iPSCs and patient immune reaction from iPSCs containing foreign antigens[15]. The microenvironment of stem cell plays an important role in the regulation of quiescence and differentiation. However, animal models do not fully encompass human microenvironments and evaluation of a biological mechanism for iPSCs engrafted for disease treatment will be important to determine therapeutic efficacy.



Generation and application of iPSCs to human disease

Cellular delivery of iPSCs to patients remains a crucial technical limitation, which has driven investigation into tissue engineering technologies. While certain therapies will suffice with single, direct implantation of iPSCs (e.g. Parkinson's disease), other approaches may require multi-site cell delivery (e.g. spinal cord injury). *Ex vivo* tissue engineering has emerged as a potential solution for artificial tissue formation and organogenesis[16]. Extracellular matrices (ECM) composed of biocompatible materials in combination with pluripotent cells and specific culturing conditions have been suggested as scaffolds for organogenesis. Technologies such as inkjet printing of collagen matrices, individual live cells, or stem cell growth factor have been used to pattern and generate custom 3D tissues.[17-19] Decellularization of endogenous cells from tissues in order to leave an ECM template where iPSCs can be added to regenerate cells has also been pursued in various organs including, heart, liver, kidney, pancreas, and intestine.[20,21] The role of hypoxia in regulating stem cells has emerged as an important facet of pluripotency and is actively being investigated.[22] Explanted microvasculature beds serving as scaffolds for large tissue formation have also been explored to overcome hypoxic environments limiting iPSC engraftment.[23]

A plethora of applications for iPSCs has been suggested, including tissue regeneration for a congenital malformations, degenerative disorders, traumas, and genetic defects. Therapeutic efficacy of hESCs has been demonstrated experimentally in animal models of spinal cord injury[24], retinal disease[25], Parkinson's disease.[26] In addition, the first FDA-approved stem cell therapy in humans was recently approved for spinal cord injury.[27] Sponsored by Geron, Phase I trials for complete thoracic injury will be performed using hESCs and have recruited the first patients in October 2010. Disease-specific iPSCs generated from patient tissues can provide new insights into the pathophysiology of various complex human diseases that currently lack effective models. Although extensive literature supports the development of stem cell therapies, results should be scrutinized diligently in order to provide the best quality of patient care.

Despite the remarkable potential for iPSCs, and the emergence of this exciting and rapidly expanding field, a variety of technical challenges remain. Historical evidence has suggested that biotechnology takes approximately 10-15 years until the development of realizable therapies.[28] While genetic engineering methods were discovered in the 1970s, FDA approval of insulin came in 1982 and was not widely available until the 1990s. Advancements in iPSC technologies will continue to develop with effort from a cadre of new researchers, challenge grants, and national initiatives. It is foreseeable that physicians in the near future will utilize stem cell therapies to treat many of the deleterious diseases currently incurable.

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