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THE PRESENT AND FUTURE

STATE-OF-THE-ART REVIEW

Translation of Human-Induced Pluripotent Stem Cells

From Clinical Trial in a Dish to Precision Medicine

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ABSTRACT

The prospect of changing the plasticity of terminally differentiated cells toward pluripotency has completely altered the outlook for biomedical research. Human-induced pluripotent stem cells (iPSCs) provide a new source of therapeutic cells free from the ethical issues or immune barriers of human embryonic stem cells. iPSCs also confer considerable advantages over conventional methods of studying human diseases. Since its advent, iPSC technology has expanded with 3 major applications: disease modeling, regenerative therapy, and drug discovery. Here we discuss, in a comprehensive manner, the recent advances in iPSC technology in relation to basic, clinical, and population health.
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Since the initial discovery that bone marrow cells possess regenerative capacity through clonal expansion, which laid the foundation for the field (1), regenerative medicine has come a long way. As a type of stem cell, these bone marrow cells have the capacity for both self-renewal and differentiation into different cell types. Earlier attempts to understand the pluripotency of the inner cell mass focused on the developmental capacity of nuclei by cloning in frogs (2), cloning in adult mammalian cells (3), derivation of mouse and human embryonic stem cells (ESCs) (4,5), and generation of ESCs and somatic cell fusion (6). Similarly, MyoD, a mammalian transcription factor, was found capable of converting fibroblasts to myocytes, which led to the concept of master regulators, transcription factors that determine lineage specification (7). Using these paradigms, subsequently discovered stem cells were classified as either adult stem cells or ESCs based on their origins and differentiation potentials. The unusual capacities of ESCs to proliferate without senescing (i.e., self-renewal) and to form all cell types of the embryo (i.e., pluripotency) made them unique and valuable resources for studying cell fate and tissue development. Initially, work on pluripotent stem cells (PSCs) was conducted using human ESCs (5); however, the requirement to destroy early-stage embryos in the process of ESC derivation made their use ethically controversial. In addition, practical considerations hindered their medical applications, because any cells or tissues generated from human ESCs by definition would be allotransplants into the recipient patient, requiring possible life-long immunosuppression therapy.

The discovery by Takahashi et al. (8,9) that a small set of reprogramming factors (e.g., Oct4, Sox2, Klf4,

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ABBREVIATIONS AND ACRONYMS

CM = cardiomyocyte

EC = endothelial cell

ESC = embryonic stem cell

iPSC = induced pluripotent stem cell

iPSC-CM = induced pluripotent stem cell-derived cardiomyocyte

PSC = pluripotent stem cell

RPE = retinal pigment epithelial

and c-myc; OSKM) can induce nuclear reprogramming of mouse (8) and adult human cells (9) to pluripotency became a landmark development in regenerative medicine. Termed *induced pluripotent stem cells* (iPSCs), they promised a source of therapeutic cells free from the ethical issues or immune barriers of human ESCs while retaining similar properties, such as self-renewal and pluripotency. Since that initial discovery, a number of advances have been introduced to enhance both the efficiency of iPSC production and the safety of the resultant lines. These improvements include the use of chemical agents to enhance efficiency (BIX-01294, valproic acid, RG108, AZA [5-aza-2′-deoxycytidine], dexamethasone, TSA [trichostatin A], and A-83-01) (10,11), use of alternative cell sources for reprogramming (embryonic, fetal, and adult fibroblasts; neural stem cells; adipose stem cells; keratinocytes; and blood cells) (12–14), use of various factors that could replace the reprogramming factors (15,16), use of vectors that can be excised from the genome (17,18), use of "nonintegrating" vectors (19,20), supplementation of reprogramming factors with microribonucleic acids (21), use of recombinant proteins/peptides to reprogram somatic cells (22,23), and most recently, a purely chemical approach (24).

With the concerted efforts of the academic community, the past decade has seen a tremendous push toward the efficient generation of safer PSCs, with the hope that one day iPSCs could be used for regenerative medicine in the clinic (25). Although halted temporarily, the clinical trial using iPSC-derived retinal pigment epithelial cells (iPSC-RPEs) for treatment of macular degeneration shows how far we have come in developing clinical-grade stem cells (26). In the short run, iPSC technology will likely offer more avenues to understand the pathophysiology of diseases and discover new therapeutic molecules. Specifically, for disease modeling, iPSCs can be generated from patients who carry certain genetic mutations and then differentiated into disease-relevant cell types, such as cardiomyocytes (iPSC-CMs) (27–29). Similarly, iPSC-derived cells can then be subjected to high-throughput screens to discover new therapeutic small molecules or conduct drug toxicity assays. Lastly, iPSC technology might enable personalized therapies (i.e., cell or tissue replacement without the need for immunosuppression, and use of drugs tailored according to each patient's genes, environment, and life-style). This is *precision medicine*, an initiative with the intent to cure each patient by taking into account his or her unique genetic makeup (30). The goal is to understand the complex mechanism of diseases so that made-to-order treatment plans could be designed for each patient based on their condition. In this review paper, we aim to highlight the promise of iPSCs for expanding basic science research and generating novel therapeutic agents for clinical and public health applications.

iPSC TECHNOLOGY: EMERGING CONCEPTS

The greatest hallmark of iPSC technology lies in its simplicity and reproducibility. Although the past decade has shown great improvement in making iPSCs safer and more efficacious, which is critical for pushing the technology toward clinical application, the mechanisms involved in efficient generation of iPSCs are just emerging. These evolving concepts raise important fundamental questions that will be discussed in the following sections.

HOW CAN A FEW TRANSCRIPTION FACTORS TURN BACK THE CELLULAR CLOCK? Many studies have tried to address this question, but the general consensus is that the initial activity of the core pluripotency genes (OSKM) has a snowball effect that results in simultaneous activation of the entire endogenous network of pluripotency genes and inhibition of lineage-specific genes within the reprogrammed somatic cells. The initial phase of reprogramming is associated with cells undergoing metabolic changes and genome-wide alterations in histone marks and methylation, followed by a late maturation phase that causes defined changes in nuclear structure, the cytoskeleton, and signaling pathways (31,32). Indeed, by looking closely at these mechanisms, researchers can now obtain nearly perfect iPSCs by clearing previous roadblocks to reprogramming (33).

ARE THESE iPSCs THE SAME AS ESCs? An important question that comes up repeatedly is whether iPSCs have the same genetic and epigenetic landscape as ESCs, and whether differentiated cells (e.g., CMs) from these 2 types of stem cells behave similarly. Despite several previous studies suggesting differences in gene expression or deoxyribonucleic acid (DNA) methylation between iPSCs and ESCs (34–36), the majority of iPSC and ESC clones are largely indistinguishable (37–39). More recent reports suggest that both cell types have identical molecular and functional characteristics, with variations in their genetic backgrounds (i.e., different donors for iPSCs and ESCs) accounting for most of their regulatory differences (40).