Current Research in Translational Medicine 64 (2016) 91–96

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General review

Human induced pluripotent stem cells: A disruptive innovation

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ARTICLE INFO

Article history:
Received 16 February 2016
Accepted 8 April 2016
Available online 25 May 2016

Keywords:
Stem cells
Induced pluripotent stem cells
Cell reprogramming
Pluripotency
Trans-differentiation
Regenerative medicine

ABSTRACT

This year (2016) will mark the 10th anniversary of the discovery of induced pluripotent stem cells (iPSCs). The finding that the transient expression of four transcription factors can radically remodel the epigenome, transcriptome and metabolome of differentiated cells and reprogram them into pluripotent stem cells has been a major and groundbreaking technological innovation. In this review, we discuss the major applications of this technology that we have grouped in nine categories: a model to study cell fate control; a model to study pluripotency; a model to study human development; a model to study human tissue and organ physiology; a model to study genetic diseases in a dish; a tool for cell rejuvenation; a source of cells for drug screening; a source of cells for regenerative medicine; a tool for the production of human organs in animals.

1. Introduction

Pluripotency is the capacity of a cell to differentiate into any cell type. In human cells, this feature is observed only in the blastocyst inner cell mass (ICM), during a very short time-window in early embryo development. Remarkably, it has been possible to 'freeze' this very transient developmental feature by identifying the culture conditions required to maintain pluripotency in vitro. This allowed the derivation of embryonic stem cells (ESCs) that self-renew indefinitely in a Petri dish, while maintaining their capacity to differentiate into all varieties of cells that form human tissues [1,2]. ESCs are pluripotent stem cells (PSC) and have opened the door to many applications, including mimicking normal tissue development in vitro or producing differentiated cells for regenerative medicine. However, ESCs have two main drawbacks. The derivation of human ESC lines is hampered by ethical issues concerning the destruction of human embryos. Moreover, it is exceedingly difficult to derive ESC lines with a specific genotype and almost impossible to obtain an autologous ESC line from a patient.

Normal human development entails the progressive specialization of undifferentiated cells into differentiated, mature cells. This process is tightly regulated by various transcription factors and non-coding RNAs as well as epigenetic remodeling. The inherent irreversibility of differentiation is required for maintaining the delicate architecture of multicellular organisms, and is schematized by Waddington (Fig. 1). However, somatic cell nuclear transfer (SCNT) studies have demonstrated that the epigenetic status of a differentiated cell can be reversed, even in mammals [3,4]. Similarly, the cell fate of a differentiated somatic cell can be brought back toward pluripotency upon fusion with one ECS [5]. Starting from these and other findings, Shinya Yamanaka and Kazutoshi Takahashi demonstrated that the transient overexpression of only four transcription factors (OCT4, SOX2, KLF4 and cMYC) can profoundly modify the transcriptome, epigenome and metabolome of differentiated cells and reprogram them into induced pluripotent stem cells (iPSCs), a new PSC type [6–8]. These results were rapidly reproduced in several other laboratories [9–12] and Shinya Yamanaka was awarded the Nobel Prize of Medicine in 2012 for this discovery [13]. Although there have been discussions on whether iPSCs are equivalent to ES cells and some epigenetic and genetic defects have been detected in iPSCs (depending on the reprogramming protocol used), the emerging

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http://dx.doi.org/10.1016/j.retram.2016.04.001
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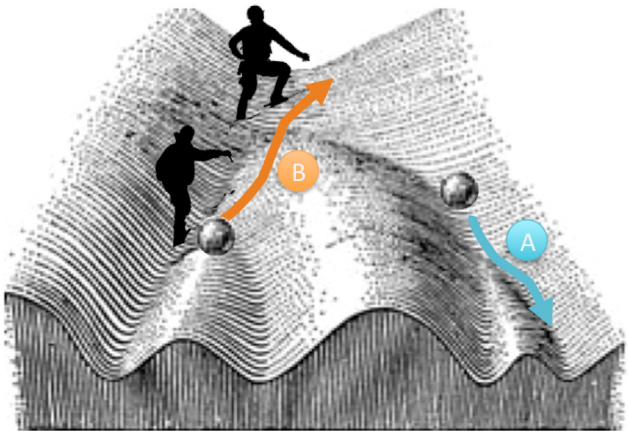

Fig. 1. Applications of the iPSC technology. The Waddington's diagram revisited [96]. A. Cell differentiation is represented by a metal ball rolling down a valley that branches into smaller valleys. This illustrates the irreversibility of the differentiation process during development and adulthood whereby a differentiated cell cannot spontaneously dedifferentiate (roll upwards) or trans-differentiate into another cell type (roll to an adjacent valley). B. Cell reprogramming is the process by which a specialized cell is de-differentiated into a pluripotent cell at the summit of the Waddington diagram.

agreement is that iPSCs are a good model to study pluripotency and may be identical or nearly identical to ES cells [14–17]. This year will mark the 10th anniversary of this discovery. The aim of this review is to discuss the most important present and future applications of iPSCs (Fig. 2).

2. Understanding cell fate control

The cell phenotype is remarkably stable despite all environmental perturbations and stochastic variability in gene expression. Hence, after commitment and differentiation, the identity of a tissue is definitive and robust. This stability is absolutely necessary. One can easily imagine the catastrophe if skin cells, for instance, could easily turn into blood cells or vice versa! To avoid this, the cell epigenome and transcriptome – including miRNAs – can withstand random perturbations of their milieu and maintain their identity. This is also true during development where differentiation leads to stereotyped phenotypic outcomes, a process termed canalization [18]. It was initially thought that differentiation was irreversible in mammals [19], but SCNT studies demonstrated that differentiation could be completely reversed also in mammals. Compared with SCNT, cell reprogramming and iPSC generation offer a more convenient tool to manipulate and study cell fate control. Currently, much effort is focused on unraveling the precise molecular mechanisms underlying cell reprogramming, ultimately to understand cell fate control [20–24].

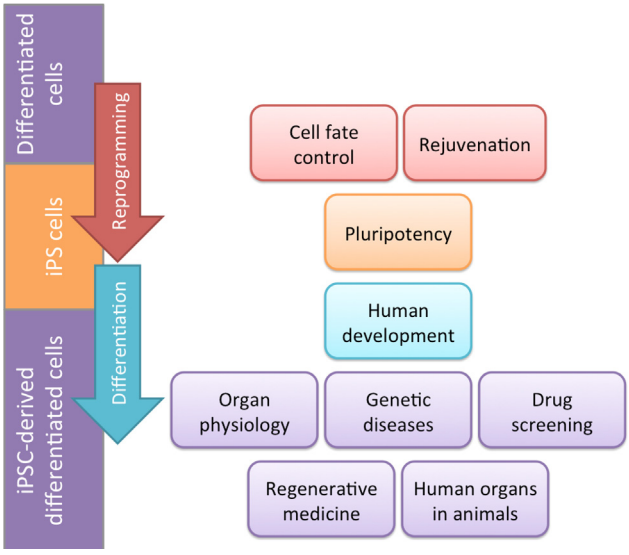

Fig. 2. Applications of the iPSC technology. The basic research and medical applications of human iPSCs can be divided in nine categories: (1) cell fate control, (2) cell rejuvenation, (3) pluripotency, (4) organ development, (5) organ physiology, (6) genetic diseases, (7) drug screening, (8) regenerative medicine, (9) human organs in animals. These categories are depicted as boxes displayed side by side with the mains steps of iPSC generation and differentiation: reprogramming, pluripotency maintenance, differentiation and differentiated cells/tissues.

Some earlier reports showed that a specialized cell type could trans-differentiate into another one upon expression of specific transcription factors [25,26]. Accordingly, iPSCs can be seen as the ultimate demonstration of cell fate plasticity through modulation of transcription factor expression. For this reason, the iPSC technology has significantly boosted the field of trans-differentiation directly [27] (transient expression of the "Yamanaka's cocktail" of transcription factors favors trans-differentiation) and indirectly, by changing the cell fate paradigm [28,29].

Moreover, the iPSC technology also contributed technically to facilitate and optimize cell identity manipulation. The initial iPSC lines were obtained by transient expression of transcription factors from murine retroviruses [6,7]. Many improvements and/or modifications of the initial protocol have been described. For example, the use of non-integrated vectors, particularly Sendai viruses, is now the preferred technique. In the future, this improvement could be applied also to the field of trans-differentiation. Techniques that avoid the use of viruses have also been developed for efficient iPSC generation, such as the use of synthetic mRNAs [30], cell-permeant proteins [31] or small molecule compounds [32,33]. Again, such approaches may be transferred to the field of trans-differentiation.

3. Cell rejuvenation

Human iPSCs can be obtained from somatic cells of aged donors and even centenarians, and, importantly, the crucial markers of ageing are reversed by cell reprogramming [34,35]. For instance, telomere length in fibroblasts derived from aged donors is extended after cell reprogramming. Even cell senescence can be reversed, as indicated by the disappearance of senescence-associated heterochromatic foci (SAHF), inhibition of p53/p21CIP1 and p16INK4A/pRB activation, disappearance of senescence-associated β-galactosidase (SA-β-Gal) activity and restoration of cell proliferation [34]. In the field of ageing, the possibility of extensively erasing markers of ageing is a major step toward the goal of rejuvenating tissues or even individuals [36,37].

Moreover, iPSCs are a unique tool to study age-related diseases in vitro [38]. For instance iPSCs have been used in the study of laminopathy-associated lamin A mutations and telomere diseases [39–41].

4. Studying pluripotency

As iPSCs are nearly identical, if not identical to ESCs, they can be used to study pluripotency instead of ESCs, thus circumventing the difficulties and ethical concerns concerning the donation/use of human embryos for research. Indeed, iPSCs have replaced ESCs for assessing chromatin modifications [42,43], nuclear genome organization [44], metabolome [45], cytoplasmic complexity [46], early human development [47] and for devising new differentiation protocols or studying organ development (see below).